

**On improving early lung cancer detection  
and localization by automated image  
cytometry and autofluorescence  
bronchoscopy**

**- A case finding study**

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## II

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# **1. Introduction:**

## **1.1. Background:**

The prognosis and survival rate of a patient with non-small cell lung cancer (NSCLC) depends mainly upon the tumor stage at the time of diagnosis. In spite of significant advancement in operative techniques, intensive-care medicine, oncology and pulmonary medicine in the last thirty years, the prognosis of lung cancer has remained virtually unchanged (53). *Ries* et al in 1983 and the American Cancer Society in 1986 pointed out that the five-year survival rate for all patients with diagnosed lung cancer is lower than 15% (67, 5). Even if we only consider the most promising stage (I A) for surgical resection (15% of all first diagnosed lung cancer), five-year survival rate of not more than 61% could be achieved (56).

On the other hand, over 90% of patients with early lung cancer (carcinoma-in-situ or micro-invasive cancer) can be cured by surgery or photodynamic therapy (22, 33). In this radiologically occult stage, the diagnosis can be established by sputum cytology, (10, 18, 72) which is a non-invasive technique. (11)

The Automated Image Cytometry (AIC) for quantitative measurement of DNA content and structure of exfoliated respiratory tract cell nuclei is considered one of the most sensitive tools developed in the last few years. AIC of bronchial wash in suspected lung cancer patients had successfully detected malignant changes with a sensitivity of 90% and a specificity of 84% (54). Being automated, AIC lends itself to large-scale screening for detection of occult lung cancer.

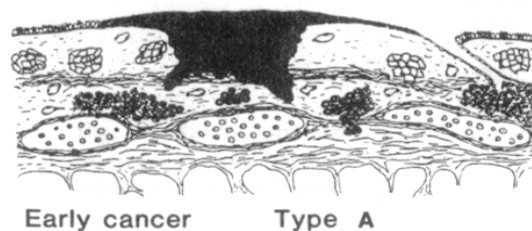
Effective treatment for a centrally located Early Lung Cancer (ELC) lesion can only be implemented by localization of the lesion itself. Localization will be improved by involvement of new technologies as Autofluorescence bronchoscopy (AF). Tissue autofluorescence was found to differentiate normal mucosa from dysplastic or carcinomatous bronchial mucosa without the need of exogenous sensitizers (47). Early results proved the superiority of combined White Light Bronchoscopy (WLB)/ Autofluorescence bronchoscopy (AF) in comparison to WLB alone with a sensitivity of 86,4% to 31,8% respectively in localizing moderate dysplasia and worse lesions, yielding a relative sensitivity of 2,7 (42).

## **1.2. Cytopathological changes of early lung cancer**

In an attempt to improve the poor survival rates of lung cancer, therapeutic strategies require a deeper understanding of the formation and progression of the disease i.e. studying the different stages of oncogenesis.

### **1.2.1. Definition of early lung cancer:**

Infiltrating growth of tumor is limited to the different layers of the bronchial wall, which means the tumor tissue does not exceed the outer tunica fibrocartilaginea, adjacent lung tissue is not infiltrated. According to the definition, an invasion of lymph vessels, pleura and lymph nodes must be excluded (17) (*Fig. 1*).



*Fig.1.* Bronchial early cancer (58)

**Microinvasive carcinoma:** is described as a few millimeters of basement membrane invasion but not involving the muscle or cartilage.

**Definition of Carcinoma in Situ (CIS):** This includes malignant cellular changes in the full thickness of the mucosa but an intact basement membrane (21). CIS is usually squamous cell carcinoma.

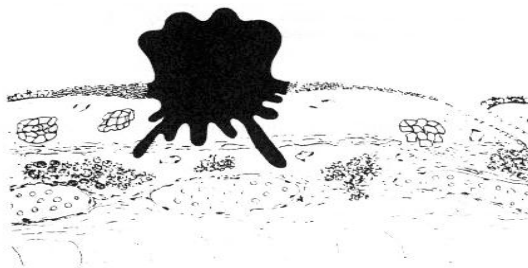
### **1.2.2. Endoscopic appearance of early lung cancer:**

In 1994, *Akaogi* and Coworkers studied the relationship between endoscopic criteria of 44 resected, roentgenographically occult, early LC lesions and the degree of histologic extent of the tumor. According to the endoscopic and macroscopic findings, the lesions were divided into three types (3):

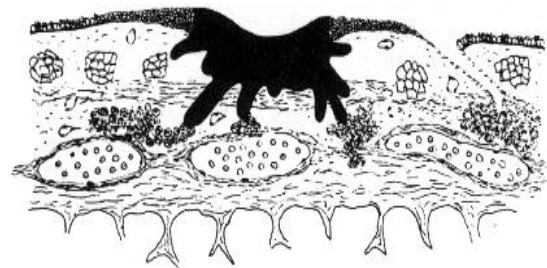


- 1- **Polypoid or nodular (PN):** The PN type was a polypoid or a well defined nodular lesion locally protruding from the surface of the bronchus (*Fig. 2 A.*)
- 2- **Flat spreading (FS):** The FS type had a non polypoid, but usually a rather thickened appearance of the bronchial mucosa with a slightly rough surface, chiefly resulting in thickening of the bronchial spur. Also, It is characterized by paleness, redness, microgranularity or loss of luster in the surface mucosa (*Fig. 2 B.*).
- 3- **Mixed Type:** The mixed type was a protruding nodule surrounded or accompanied by an irregular thickening of the bronchial surface.

It was shown that FS type is the most common growth pattern in the central bronchus (19/33) and the only one peripherally (11/11).



**Fig. 2.A.** Scheme of Polypoid type of early LC (58)



**Fig.2 B.** Scheme of Flatty Spreding type of early LC (58)

PN and mixed types were found of the same proportions (7/33 each) and only centrally.

Lesion type and size were correlated to the depth of bronchial invasion and LN involvement. So, endoscopic criteria of small endobronchial lesions could disclose information on the stage of the disease. Central PN lesions smaller than 10 mm and central FS lesions less than 15mm in greatest dimension were likely to be early lung cancer.

### **1.2.3. Outcome of Preneoplasia:**

There is good evidence to indicate that the natural history of lung cancer in these very early stages may extend over a period of years before the tumor becomes radiographically demonstrable. However, the extent to which preneoplastic lesions precede one another in time and their precise outcome remains largely unknown (83). On the basis of cytological data, studies showed that approximately 11% of moderate dysplasia and 19% to 46% of severe dysplasia would progress to invasive cancer. (8, 68).

Also, *Saccommanno* et al. in 1974 recorded the average times of transition from carcinoma *in situ* to invasive squamous cell carcinoma at 2.5 years, ranging from 0.6 - 6.2 years (71). Meanwhile, many authors reported the regression of some preneoplasias and even CIS after withdrawal of the carcinogenic insult-usually cigarette smoking (75). Finally, and in a recent study conducted by *Venmans* et. al in 2000, they followed up their diagnosed cases of CIS and described that 78% of these lesions progressed into invasive cancer recommending the mandatory treatment of these lesions (81).

### **1.3. Importance of endoscopic early lung cancer detection:**

Experienced bronchoscopists can detect 29% of carcinomata-in-situ and 60% of micro-invasive carcinoma (*Woolner* 1983). A more sensitive method for detection and localization of early lung cancer is needed urgently as well established methods for early treatment of CIS and micro-invasive cancer (19, 28) are available even for functionally inoperable patients. ND-YAG Laser, electrocautery, photodynamic therapy and cryotherapy are all useful tools for such a therapy.

Meanwhile, a clear relation between the surface area of the lesion and the success of treatment was observed by *Hayata* et al in 1993. A lesion < 1cm will be eradicated by photodynamic therapy in 97.8%, while in lesions > 1cm, cure will be achieved in only 42.9% (33). So, the early diagnosis directly influences the success of treatment. As improvements in image resolution and quality have not enhanced detection of early lung cancer, technical developments turned to getting help from other optical qualities e.g. the native fluorescence characteristics of the endobronchial epithelium.

### **1.4. The screening problem:**

Two screening methods were applied trying to improve the early diagnosis and respectively the prognosis of lung cancer, plain chest x-ray and sputum cytology. Sputum is collected for three days in a special conserving solution, a technique suggested by *Saccommanno* for a better diagnosis rate of lung cancer. Material subsequently stained according to papanicolaou stain and microscopically examined. These methods were tried in large studies (30, 55). Study end point was lung cancer mortality reduction. The Mayo clinic lung project (4 monthly chest x-ray and sputum cytology in the test group versus once yearly clinical examination in the control group) recruited 9211 male smoker in their project.

Although they could improve the percentage of resectable lung cancer cases from 32% of the diagnosed cases to 46%, the difference in mortality rate between the examined group and the control group remained unchanged. Similarly the Memorial Sloan-Kettering and the Johns Hopkins projects (once yearly chest x-ray in both groups plus 4 monthly sputum cytology in the first group only) showed no change in mortality rate.

The conclusion from the above-mentioned data was that screening sputum cytology did not decrease the mortality rate. The overall sensitivity of sputum cytology was 22% and 48.7% (30). Early lesions of non-small cell lung cancer in John Hopkins study were sputum positive in 15% only. A lot of peripheral lesions were not represented in sputum. The specificity of sputum cytology is high (26). From 81 cases with stage I tumor in the Mayo clinic lung project, 44 cases were x-ray positive (peripheral adenocarcinoma), 30 cases were sputum cytology positive and radiologically occult (central squamous cell carcinoma), and 7 cases were radiologically evident and sputum positive. Sputum cytology and chest x-rays complement each other in detecting early lung cancer.

To summarize, it was found that the value of chest x-ray in screening is debatable, no added advantage for using sputum cytology could be proved and both methods did not effectively reduce the mortality rate. Screening for lung cancer was abandoned as a waste of resources (4, 27).

## **1.5. Automated Image Cytometry (AIC)**

### **1.5.1. Background:**

DNA cytometry has gained wide acceptance in pathology and cytopathology as a means to obtain objective information concerning the diagnosis and grading of human cancer (12). With this technique, cells are stained specifically for DNA, and digital images of microscope fields are acquired, typically using a CCD camera. Computers are used to process the digital images and to perform a wide variety of Nuclear feature measurements, such as the size, shape, and DNA content, as well as features describing the spatial distribution of chromatin within the nucleus (24).

One of the most widely applied image cytometry measurements is that of DNA content of the nucleus which is accepted by an international

consensus group (1). Using this new technique, we can detect not only the DNA amount inside the nucleus but also the chromatin structure and distribution. As a rule, the higher the malignancy grade of the tumor, the higher will be the DNA content, its uneven distribution and the number of aneuploid cell nuclei. The genetic basis for the relevance of tumor aneuploidy measurements has been well established through cytogenetic studies of tumor cell populations (29). In these studies, correlation was demonstrated between cytometric measurements of aneuploidy and gene or chromosome amplification processes. In addition, karyotypic instability has been shown to have dramatic effects on DNA content distribution in tumor cell populations.

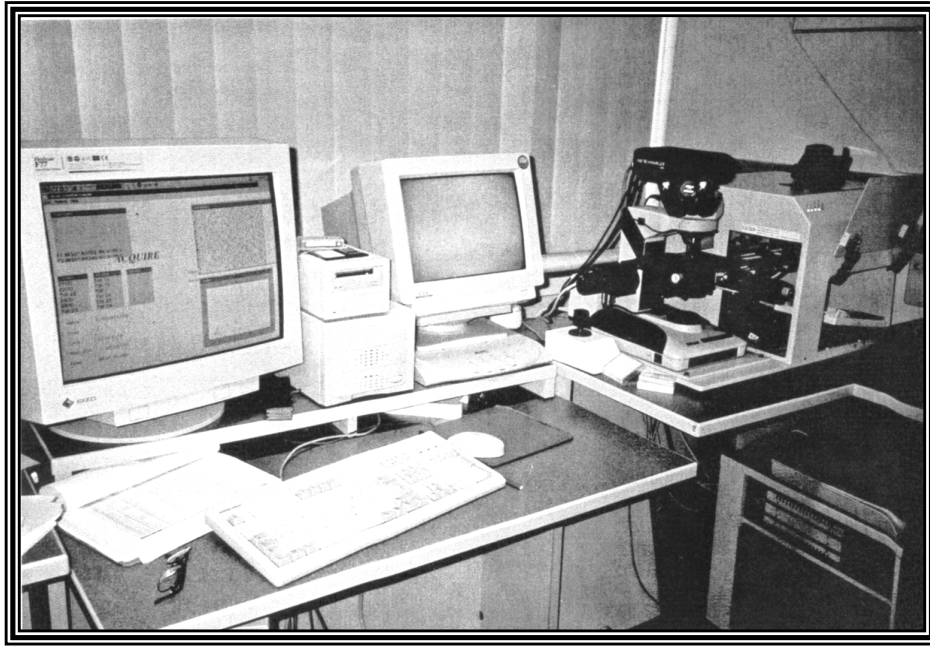
However, the DNA content of exfoliated cells from the respiratory tract can be affected by a number of factors that must be considered in the differential diagnosis of either sputum cytology or cytometry. Some of these are the replication rate, (polyploidal) nuclei, nonspecific effects of chemotherapy and radiotherapy, vitamin B12 deficiency, autolysis, necrosis and viral infection.

The Cyto-Savant® is a promising device developed recently in corporation between the British Columbia Cancer Agency and Oncometrics Corp. Vancouver BC, especially for gynecological screening examinations (31) **Fig 3**.

### **1.5.2. Method of Automated Image Cytometry:**

The prerequisites of AIC was determined in the consensus report of the European society for analytical cellular pathology task force on standardization of diagnostic DNA image cytometry (12). An automated cytometer device requires a high resolution level by provided efficient focus range. DNA content and structure of a nucleus must be measured independently from a monolayer slide without any qualitative or quantitative failure.

The device (Cyto-Savant®) feeds the microscope automatically with slides. Nuclei are focused by a high spatial and photometric resolution. For every object, the program will calculate 114 features that will identify and eliminate artifacts and then will classify cells into groups of lymphocytes, granulocytes, epithelial nuclei, alveolar macrophages and abnormal or suspicious nuclei.



**Fig. 3.** Photo of Cyto-Savant<sup>®</sup> (from right to left: automatic slide supplier, Microscope with a connected CCD camera, monitor for microscope, magnetic disk operator and the analysis monitor)

A representative number of leucocytes will be taken to normalize the features as regards to the different staining characteristics between different batches of slides. Then with the use of trainable classifiers, will the different nuclei be arranged in different groups and their picture will be projected on a screen. From every group, the mean and standard deviation of every feature will be calculated and tabulated. Then a distribution histogram of different feature combinations will be obtained.

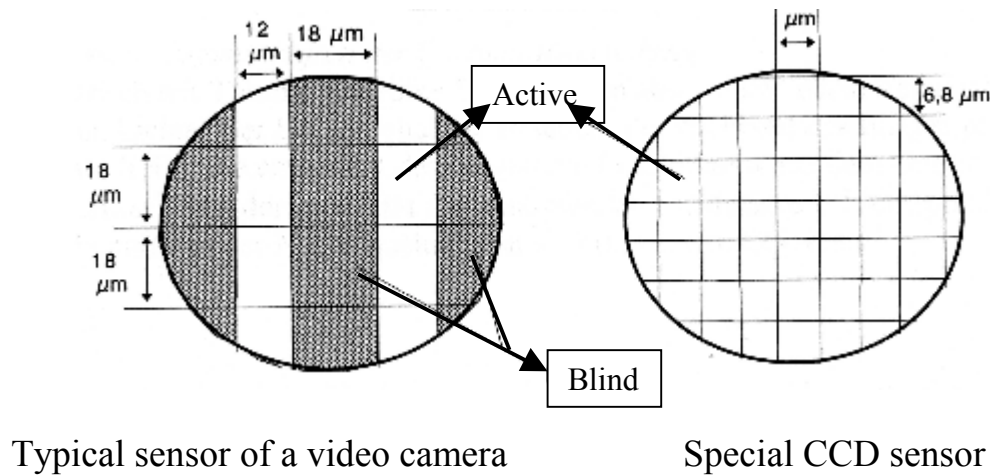
### **1.5.3. Technical specifications:**

A special CCD (charge couple device) camera is used. It observes the whole field at the same time with a picture frequency of 20 MHz without blind spots. (**Fig. 4**) is an example for such a camera (Microimager 1400), Xillix Technologies Corporation.

### **1.5.4. Cell tracking:**

In the current version, the cytometer identifies and analyses up to 50.000 objects in 30 minutes. About 2000 cells (1000 epithelial cells, 200 suspicious nuclei with a DNA index of more than 1.25 and less than 2.5 and up to 100 highly suspicious ones with a DNA index of more than 2.5) were collected and stored. Along with the epithelial cells, up to 200

lymphocytes, polymorphonuclear and eosinophil granulocytes were identified and 100 alveolar macrophages each.



**Fig.4.** Difference between CCD sensors and conventional video cameras: normal video image has blind spots, CCD has the “full-fill” factor, i.e., no blind spots.

The standard value for the integrated optical density (IOD) of epithelial cells was 110. For each of the 2000 cells, digital values of all features were calculated and stored. Coordinates of all cells were stored and suspicious cells could be revisited interactively. The DNA amount of normal epithelial nuclei, referred to as 2c value or euploidy value, is calculated from a representative group of lymphocytes.

Three parameters were calculated from the DNA value of epithelial nuclei: the rate of 5c exceeding nuclei (5cER), the 2c-deviation index (2cDI) and the malignancy grade (MG). The **5cER** is the rate (in %) of aneuploid nuclei with a DNA amount  $> 5c$ . These are different from normal separating mitotic nuclei. Nuclei are called euploid if their DNA amount is in the range  $2c \pm 0.25c$ . **2cDI** is defined as the sum of all squared deviations of the DNA amount of all epithelial cells ( $C_i$ ) from the mean value ( $2c$ ) divided by the number of cells. This value is equivalent to the mean square from the deviation of the mean diploid value. Further gradations are based on the malignancy grade (**MG**) as the logarithmically transformed 2cDI. This procedure allows quantifying the whole spectrum of malignancy with the help of a simple score.

## **1.6. Autofluorescence Bronchoscopy (AF)**

### **1.6.1. Historical background:**

The concept of fluorescence detection, as an aid for diagnosis of malignancies, has intrigued the minds of many since the beginning of the twentieth century. **Policard** observed red fluorescence under ultraviolet light as early as 1924 in an experimental model of mouse sarcoma (65). The trial of intra-operative differentiation between normal and tumor tissue with the help of an ultraviolet operation lamp was an early application of this concept. Subsequent investigations showed that this red fluorescence was due to the presence of porphyrins.

In 1933, **Sutro** and **Burman** observed that when surgically excised breast tissue was exposed to ultraviolet Wood's light, normal breast tissue fluoresced green while breast cancer tissue fluoresced purple and this could be helpful in detecting resection borders (74). It was also observed by **Ronchese** in 1954 that advanced cancer of the breast, mouth or skin emits a bright red fluorescence upon illumination by Wood's light (69).

In 1964, **Lipson** at Mayo clinic synthesized hematoporphyrin derivative (HpD) (52). HpD was found to have superior tumor localizing properties than hematoporphyrin alone for a variety of tumors including lung cancer. The partially purified preparation is known as photofrin (proflimer sodium). Although various experimental fluorescence bronchoscopy systems have been developed to localize early lung cancer as well as to treat invasive tumors by using HpD or photofrin (20, 40), clinical application has been limited by several problems:

- a) The fluorescence yield from a small, thin CIS is often weak when compared to invasive cancers.
- b) Some areas with diffuse fluorescence and blurred margins or areas with low contrast are difficult to differentiate from the normal background. The magnitude of this contrast appears to correlate with the stage of cancer, with the more invasive tumors showing the highest contrast. (15).
- c) **Baumgartner** in 1991 proved the non-specific uptake of the fluorescent drug by inflammatory cells. He showed 27% false positive results even with a dose of photofrin that is one-fifth of that conventionally used for photodynamic therapy (9).
- d) The serious side effects of photofrin or HpD as regards skin photosensitivity. Patients who have received photofrin or HpD have to



remain out of any strong light for thirty days and sometimes for several months (25).

- e) Almost all of the fluorescent drugs are available for investigational use only. Even when these drugs become available for clinical use, they will add to the cost of bronchoscopic examination (51).

An interesting finding was the discovery that detection of dysplasia and carcinoma in-situ by fluorescence techniques can be achieved without using any drug at all. In vivo spectroscopy during bronchoscopic examination with an optical multichannel analyzer and a Helium - Cadmium Laser (442 nm) for illumination showed a significant decrease in autofluorescence intensity, predominantly in the green region of the visible spectrum in areas with dysplasia or CIS compared to normal bronchial tissue (35). The advancement in technology of screen resolution and computer data analysis in the last few years helped the development of this discovery (66). A well established, now in routine use, system was developed in association with the British Columbia Cancer Agency, the LIFE-Lung (Laser Induced Fluorescence Emission) system (Xillix)<sup>®</sup>

**Fig. 5.**

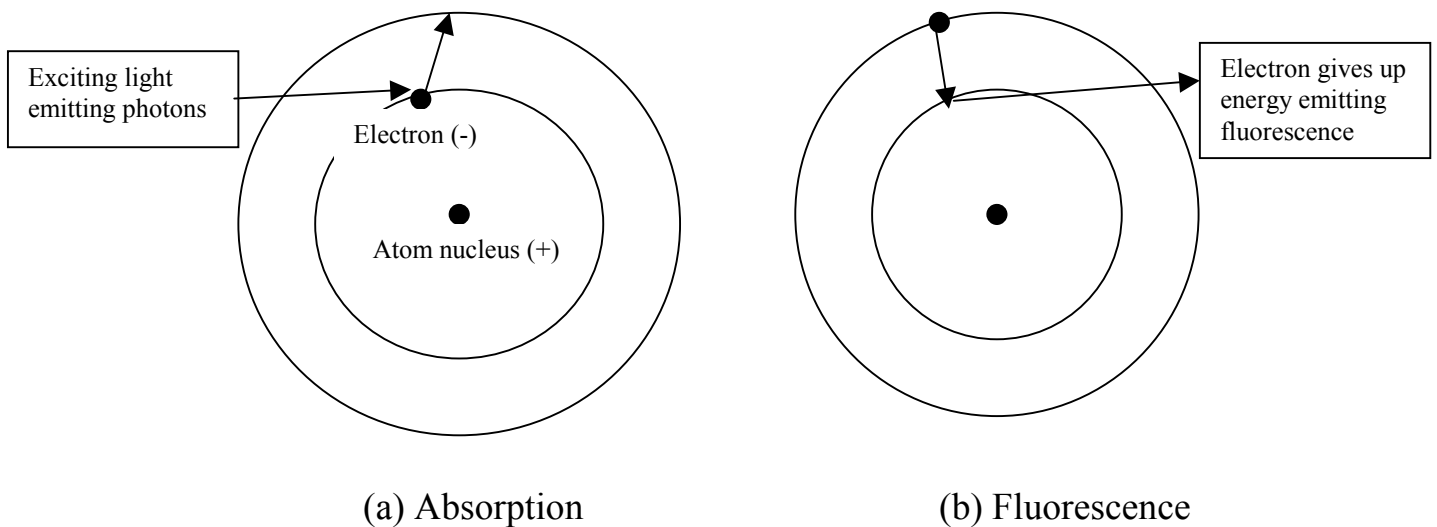


**Fig. 5.** Photo of Xillix LIFE-Lung Fluorescence Endoscopy System<sup>®</sup> (right: Imaging console integrating the CCD camera with suspension system, touch screen monitor, processor and a VCR. Left: Illumination console for He-Cd laser)



### **1.6.2. Causes of autofluorescence phenomenon:**

Fluorescence is a physical phenomenon, which appears when atoms in tissues are excited with light photons of a specific wavelength that are absorbed by electrons causing their movement to a higher energy level. The electron gives up its energy after a short time and returns to its original orbital emitting its energy as photons. This emitted light is the source of fluorescence, which is taken up and processed by the highly sensitive CCD camera (*fig 6*).



**Fig. 6.** Showing (a) absorption of photons by electrons and its movement to a higher energy level (excitation) and (b) emission of this energy in the form of photons giving rise to fluorescence.

This reaction appears in living as well as dead tissues and it has long been used as a parameter to differentiate between healthy and diseased tissues. Tissue substances emitting fluorescence are named fluorophores and tissue type and metabolic state control their localization and concentration. The following substances found in the human body act as fluorophores: (Table 1)

<b>Table 1. Fluorescing Substances in human body</b>		
Fluorescent substance	wavelength of excitation light (nm)	Fluorescent wavelength (nm)
1- Tryptophan	280	340
2- Collagen	325	380
3- Elastin	410	440
4- NADH	365	470
5- Flavin	440	520
6- Porphyrin	400	630,690

The causes of the difference in fluorescence properties is a matter of research till now. Difference in epithelial thickness, increased blood supply in malignant lesions through increased vascularisation (hemoglobin absorbs nearly all the green light), the over-production of lactic acid by cancerous cells due to increased glycolytic activity and the change in concentration and oxidation state of the fluorophores may all play a role (35, 6, 2).

Non imaging instruments have been devised primarily to allow quantitative measurements of fluorescence or fluorescence ratios in different wavelength bands. Disadvantages of the non-imaging systems are the non-precise delineation of lesions, very small lesions can be missed and in vivo calibration may take by chance a reference area of unknown dysplasia or metaplasia (45).

### **1.6.3. Instrumentation:**

#### **1.6.3.1. The LIFE<sup>®</sup> Lung system:**

Fluorescence bronchoscopy requires a bronchoscope, a light source for excitation of fluorescence and a detection system.

The bronchoscope used is a conventional white light flexible fiberscope. The light source for white light bronchoscopy is a xenon arc lamp, typically 200-1000 W. this source delivers a broad spectrum in the visible range; the accompanying infrared emission is filtered out. A white light source is probably not suitable for fluorescence bronchoscopy, because the nonspecific part of the spectrum overlaps the weak fluorescence of interest, making it impossible to detect. There are new mercury arc lamps that are coupled with small fiberoptic light guides and appropriate filters that filter out the longer wavelengths overlapping the fluorescence, which can replace more expensive laser light sources (51).

The preferred light source is a low energy 150-mW laser, emitting about 12-20 mW at the distal end of the light guide bundle. At a typical bronchoscope-to-tumor distance of about 1cm, this power is sufficient and avoids thermal effects. For excitation of autofluorescence, a helium-cadmium laser has been found suitable, emitting in the blue range at 442 nm (35, 61, 47, 50). The blue light is transmitted through the built-in fiberoptic light guide normally used for white-light illumination. It is important that the spatial distribution of the irradiance in the bronchus is

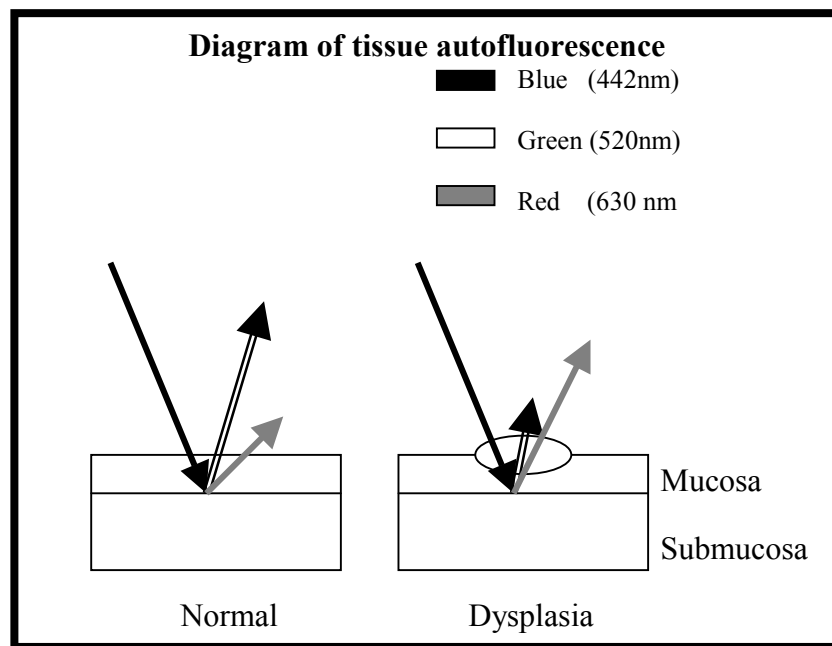
quite uniform; otherwise irregular shadows caused by non-uniformity or a projection of tissue could be mistaken for a tumor.

The detection system for imaging is critical, and various solutions have been proposed. The eye alone is not sensitive enough to detect the weak fluorescence from small, thin tumors even when they contain a fluorescent agent such as photofrin, especially considering the light losses in the bronchoscope. Theoretically, increasing the laser power and hence the excitation irradiance would increase the fluorescence intensity. A higher output laser may damage normal tissue. Instead, image intensifiers are used to amplify the weak fluorescence signal to a level that can be detected by the light-adapted eye (necessary in the bronchoscopy environment) or a CCD video camera (51).

LIFE is comprised of a low energy helium-cadmium laser as a monochrome light source (442 nm), two images intensified CCD cameras with green and red filters respectively, a computer with an imaging board, and a color video monitor. Two images at different (red and green) wavelengths are simultaneously captured in precise registration by the imaging board. When the bronchial epithelium is illuminated by the blue light, it will have its peak of fluorescence in the green wavelength (520 nm). Premalignant changes as dysplasia or CIS will emit red-brown color with a wavelength of 630 nm as seen in *fig 7*

The above mentioned filters capture the emitted wavelengths from the bronchial epithelium in the spectrum of 520 nm and > 630 nm and exclude the excitation wavelength (51).

The images are then combined and processed by the imaging board using a specially developed algorithm that allows normal tissue to be clearly distinguished from malignant tissue when displayed as a pseudocolor image on the video monitor. The captured image is displayed in real time (at video rates). The processed image can be displayed as desired, for example, normal tissue as green and tumor tissue as brown or brownish red. An abnormal area can be biopsied under direct vision for pathological confirmation.



**Fig. 7.** Fluorescence light-emitting characteristics of normal and dysplastic bronchial mucosa

#### **1.6.3.2. Other autofluorescence devices:**

Increasing interest in ELC detection has encouraged the development of more imaging AF-bronchoscopy systems with slightly modified technique. System ***D-Light*** AF (Storz, Germany), a system adapted to rigid bronchoscopy as well uses filtered Xenon instead of laser light for excitation (32). Impairment of AF can be observed either through the bronchoscope directly or with the help of a video camera. Prolonged exposure time required for the AF video camera mode leads to sequential frozen frames on the monitor. With the System ***D-Light*** as well as with the similar ***SAFE 1000*** System (Pentax, Japan) switching between WLB and AF-mode is possible without changing light source and camera. The display of AF-images with the ***SAFE 1000*** provides a continuous real time image; the system is adapted to flexible bronchoscopy (37).

#### **1.6.4. Clinical applications of AF:**

The LIFE imaging system facilitates earlier detection of lung cancer and provides physicians with more information for the management of the diagnosed lung cancer patients. Using the LIFE imaging system for the following indications will supply useful diagnostic information.

#### **1.6.4.1. Indications of AF examination:**

Patients at high risk for lung cancer are the target group for AF examinations. These are:

- 1- **Patients with previously resected for cure lung cancer:** Patients with previously resected stage I lung cancer have local recurrences or second primary lung cancers at a rate of approximately 3.6 per year (77). From what is known about the natural history of lung cancer (70), the second primary cancers that were diagnosed in the first 3-5 years after surgery were probably present as dysplasia or CIS at the time of initial bronchoscopy before surgery and were missed by white light bronchoscopy.
- 2- **In preoperative assessment of patients with lung cancer:** in order to determine the extent of endobronchial involvement and resection margins respectively.
- 3- **Patients with suspected bronchogenic carcinoma:** (symptoms, abnormal chest x-ray, positive sputum cytology or cytometry, undiagnosed pleural effusion or paraneoplastic syndrome).
- 4- **Patients with head and neck cancers:** as there is a high incidence in developing a second primary cancer not only locally but also in the lungs (62).
- 5- **Individuals at otherwise high risk of developing lung cancer:** i.e. patients who are current or past heavy smokers with positive family history of lung cancer, workers with asbestos or other relevant industrial exposure.

#### **1.6.4.2. Other potentially useful Applications:**

- 1- Delineation of the exact extent of the tumor before photodynamic therapy is of particular importance for dosimetry calculation, fiber type, total treatment sessions and to ensure complete eradication of the entire tumor (50).
- 2- Autofluorescence bronchoscopy may serve as a vehicle to educate bronchoscopists in recognizing subtle changes during routine bronchoscopy.
- 3- This method allows detection and biopsy of pre-malignant respiratory epithelium for genetic and differentiation marker studies.

### **1.6.4.3. Contraindications for AF examination:**

- 1- Patients with known or suspected pneumonia.
- 2- Patients with acute bronchitis within one month of the procedure.
- 3- Patients with white count less than 2000 or more than 20 000, and/or platelet count less than 50 000.
- 4- Patients who had received fluorescent photosensitizing drugs such as photofrin within three months of the procedure.
- 5- Patients who are on, or have received chemopreventive drugs (e.g. retinoic acid) within three months of the procedure.
- 6- Patients who had received ionizing radiation treatment to the chest within six months of the procedure.
- 7- Patients who had received cytotoxic chemotherapy agents systematically within six months of the procedure.
- 8- Other general contraindication for bronchoscopy as patients with bleeding disorders, left ventricular failure, uncontrolled hypertension, unstable angina and known reaction to topical xylocaine.

### **1.7. Aim of the study:**

The following questions will be addressed in this study:

- 1- Is automated image cytometry a sensitive tool for recognizing early malignant changes in nuclei of bronchial mucosal cells?
- 2- Is autofluorescence bronchoscopy a reliable and sensitive tool for early detection and localization of centrally located early lung cancer lesions?
- 3- Can an increase in the diagnostic rate of preneoplasia be achieved by the combined use of automated image cytometry and white light/autofluorescence bronchoscopy?

The study is conducted at the Research Institute for Diagnosis and Treatment of Early Lung Cancer (RIDTELC)<sup>TM</sup> of the Augusta Teaching Hospital in Bochum, Germany.

## **2. Patients and Methods**

### **2.1. Recruitment of patients:**

For this prospective case finding study, we recruited 119 high-risk patients from January 1999 till June 2000 in whom all initial radiological investigations for presenting complaints were negative for lung cancer. All patients submitted a sputum sample for AIC, underwent white light (WLB) and autofluorescence (AF) bronchoscopy with bronchial washings for AIC and conventional cytology (CY). Endoscopically suspected lesions were biopsied and examined histopathologically. A total of 150 examinations were performed on these patients after taking informed consent from each of them.

### **2.2. Inclusion criteria:**

- 1- Patient should be able to give an informed consent.
- 2- Patients are at high-risk for lung cancer. For example, they should have either:
  - A) Heavy smokers with a positive family history of lung cancer, occupationally exposed workers to carcinogenic substances as uranium miners, COPD with a change in symptoms as recurrent unexplained hemoptysis.
  - B) Follow up of resected for cure lung cancer to search for recurrence in resection margins or second primary.
  - C) Previous atypical cytological or cytometrical results of bronchial secretions.
  - D) Patients at otherwise high-risk for developing lung cancer for miscellaneous reasons as patients with upper digestive or respiratory tract cancers or head and neck cancers.

### **2.3. Exclusion criteria:**

- 1- Definite endobronchial tumor growth.
- 2- Acute bronchopulmonary infection with possible bronchial hyperemia and increased coughing.
- 3- Radiotherapy and or chemotherapy 6 months prior to the examination.
- 4- Interventional bronchoscopic procedures 2 months prior to the examination including biopsy as this will artificially reduce AF.
- 5- Current photosensitizing medications such as photofrin.

- 6- Contraindications of WLB in general as unstable angina, bleeding tendency, left ventricular failure, uncontrolled hypertension and allergy to local anesthesia.

#### **2.4. Investigations:**

- 1- Full history and clinical examination.
- 2- Chest x-rays and computed chest tomography if necessary.
- 3- Sputum sample for automated image cytometry examination with Cyto-Savant®.
- 5- White light bronchoscopy with Olympus BF 20 or 40, Tokyo, Japan).
- 6- Autofluorescence bronchoscopy with LIFE® system.
- 7- Bronchial washings for conventional cytology as well as automated image cytometry also with Cyto-Savant®.
- 8- Bronchial mucosa biopsies for histopathological examination or brushing for cytology were done when a preneoplastic lesion was suspected under White Light Bronchoscopy (WLB) and/or Autofluorescence Bronchoscopy (AF).

#### **2.5. Sputum collection /preparation for bronchoscopy:**

- 1- After giving informed consent for the examination, an intravenous line was introduced and topical anesthesia was applied by inhalation of 5 ml Lidocaine 4% solution for 10 minutes through an ultrasonic nebulizer.
- 2- The patient was instructed to cough as deep and as strong as possible and to expectorate about 10-15 ml into a container with 20 ml Saccomanno solution (50% ethyl alcohol & polyethylenglykol) mixed with 0.2% dithiothreitol for good sputum liquefaction.
- 3- **Bronchoscopic procedure:** in a recumbent position, intravenous sedation with midazolam was sometimes given and individually titrated to keep the patient calm and prevent excessive and frequent coughing during the examination. Such cough can traumatize the bronchial mucosa against the bronchoscope or cause petichial hemorrhages from severe Valsalva maneuver and both will reduce autofluorescence making it difficult to diagnose minute preneoplastic lesions especially for the inexperienced. The fiberoptic bronchoscope was coupled with a conventional video camera and introduced transnasally when possible, otherwise transorally under nasal oxygen supplementation and pulse oxymeter control. Careful bronchoscopy was done to prevent unnecessary mucosal trauma.



After documenting all suspicious lesions under WLB, the laser light source was connected to the fiberoptic bronchoscope and the CCD camera replaced the conventional video camera. Suspicious lesions under AF were also documented then reexamined again by WLB and documented if they were previously classified as non-suspicious. Then biopsy was taken from all suspiciously classified lesions either with one or both methods. Biopsies were taken separately using a new forceps for each lesion to prevent contamination.

## **2.6. Documentation:**

The whole examination during WLB and LIFE was video documented. Freeze frames of all suspect lesions were stored in WLB and AF together with the endoscopic evaluation. AF suspicious lesions overlooked in WLB were captured as freeze frames but classified as non-suspicious.

## **2.7. Chronology of specimens collection:**

After completion of documenting all findings, bronchial washings from central airways were the first specimens to be taken. Then all endoscopically suspicious areas with either one or both methods were biopsied and sent for histopathological examination. All obviously invasive tumors, which were radiologically occult, were biopsied for diagnosis but excluded from the study. If there were no suspicious lesions, no biopsies but bronchial washings were taken for cytology and automated image cytometry to search for peripheral early lung cancer lesions that could not be reached by the bronchoscope as well as centrally located lesions that may be missed from both endoscopic methods.

## **2.8. Technique of endoscopic specimen collection:**

Bronchial washings were done by instilling 4 x 10ml isotonic saline on main carina and suctioning at least 20ml from both sides of the bronchial tree.

The washings were equally divided into two test tubes; one containing 10 ml of 50% alcohol as a fixative for cytological examination and the other with 20 ml Saccomanno solution for automated image cytometry. Suspicious lesions were biopsied either with a toothed biopsy forceps (Olympus FB 19 C) or by brush (Cellebrity 1.7mm, Microvasive). Biopsied tissues were either collected in 4% formaline solution for staining with Hematoxylin & Eosin and with Giemsa stain for

histopathological examination or spread on a slide, air dried and stained according to Papanicolaou for cytological examination.

## **2.9. Evaluation criteria:**

### **2.9.1. Endoscopic Image Classification:**

The examiner's impression for the image diagnosis of a suspicious lesion was classified and documented as follows:

**Class I:** for normal bronchial mucosa or benign unsuspected lesion as inflammatory changes.

**Class II** for suspected preneoplastic or early carcinomatous lesion.

**Class III** for definite invasive tumor growth. Class III was totally excluded from the analysis *Fig 10*.

#### **a) White light bronchoscopic image classification:**

**Class I** for normal or diffuse redness with or without edema of bronchial mucosa (inflammation), granuloma, angioma, scarring, telangektasia and trauma *Fig. 8*.

**Class II** for localized redness or paleness with well-circumscribed edema, thickening of carina, loss of luster, irregular mucosal surface of small polyps.

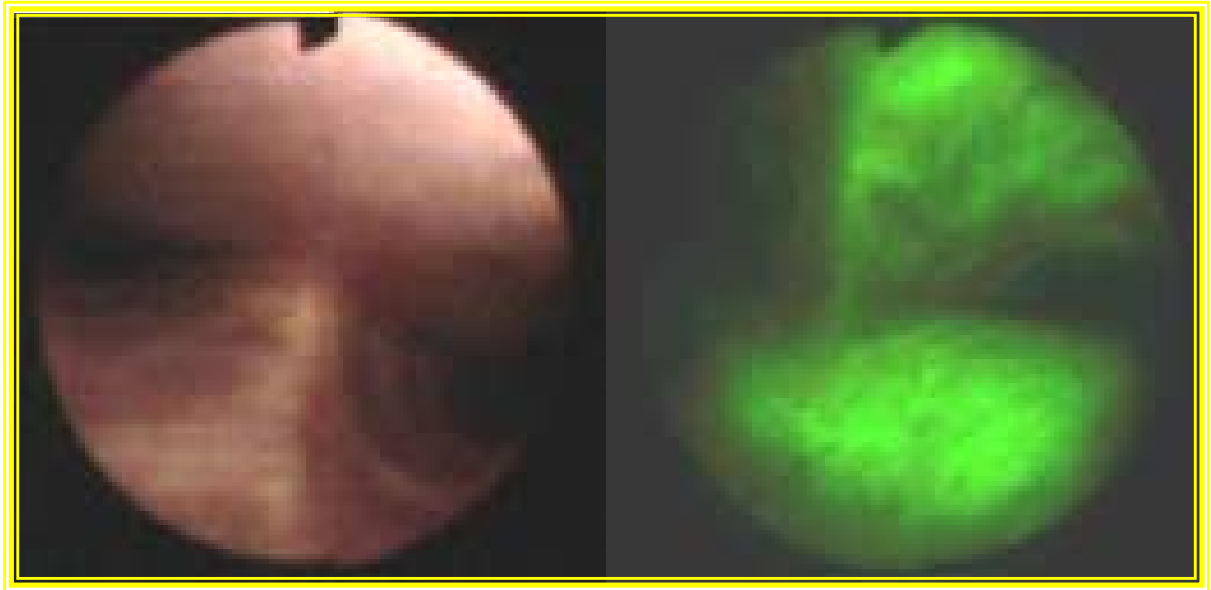
**Class III** for definite endobronchial tumor growth, mucosal infiltration with loss of normal structure, bronchial wall stiffness, vulnerability and bronchial stenosis *Fig. 10*.

#### **b) The corresponding classification in autofluorescence mode was:**

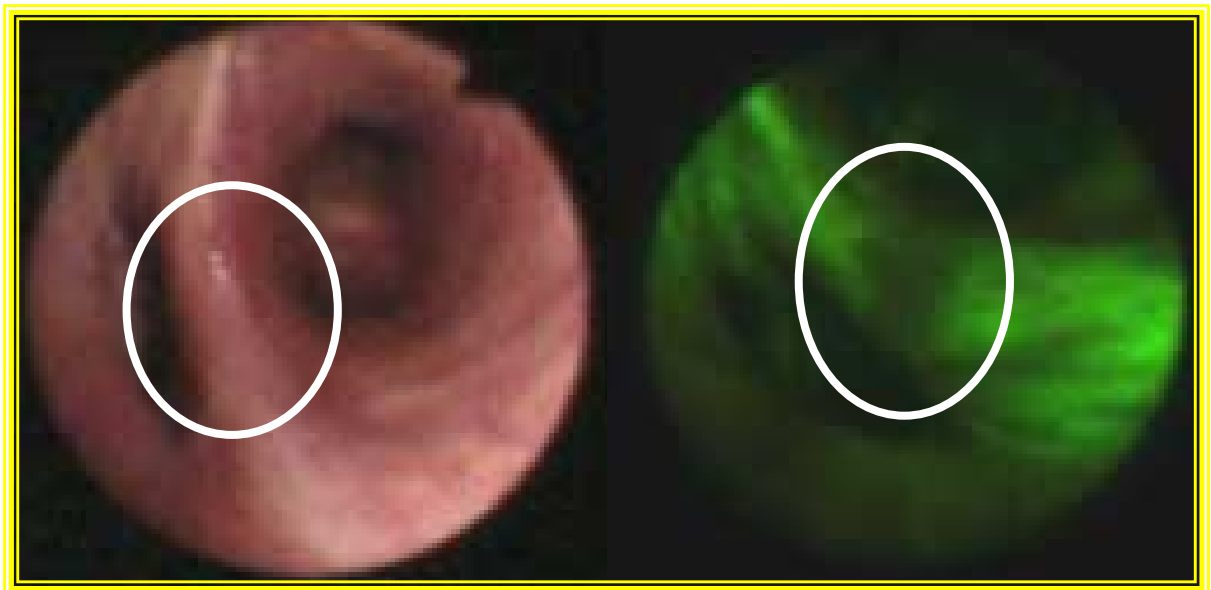
**Class I** for bright green mucosa, well-demarcated dark brown in hematoma, or superficial vessels. *Fig 8*.

**Class II** for well-demarcated homogenous, dark brown area *Fig 9*.

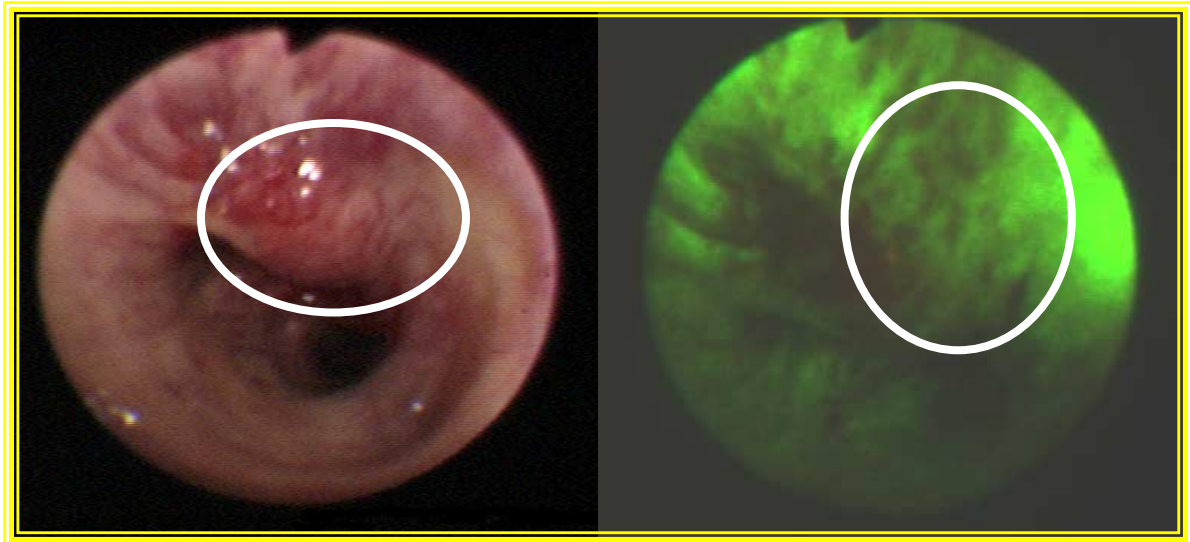
**Class III** for well-demarcated, dark brown color may be with areas of exaggerated green fluorescence (necrotic tissues or destroyed cartilage) *Fig 10*.



**Fig. 8 Normal main carina under WLB and AF modes**



**Fig.9 Severe dysplasia of Rt. upper lobe carina**



**Fig. 10 Tumor growth in Lt. Upper Lobe Bronchus**

### **2.9.2. Histopathological Classification of biopsies:**

The histological diagnosis of mucosal structure was done according to the WHO criteria 1981 (Histological typing of lung tumors). In: WHO (Ed.): International histological classification of tumors (1981).

- 1.0 Normal
- 2.0 Inflammation
- 3.0 Hyperplasia
- 4.0 Mild dysplasia
- 5.0 Moderate - Severe dysplasia
- 6.0 Carcinoma in situ (CIS)
- 7.0 Microinvasive carcinoma
- 8.0 Invasive carcinoma
  - 8.1 Squamous
  - 8.2 Adeno
  - 8.3 Large cell
  - 8.4 Small cell
  - 8.5 Other
- 9.0 Unsatisfactory specimen.

For final pathologic diagnosis, the nine point's scale was converted into a three-points. Codes 1-4 were considered negative, codes from 5-8 were considered positive and code 9 was labeled 'not evaluable'.

### **2.9.3. Cytological grading of Bronchial Secretions:**

For cytological diagnosis is a modified classification of Papanicolaou was applied:

- Pap 0: not representative.
- Pap I: Normal.
- Pap II: benign diagnosis as inflammation.
- Pap III: cellular proliferation with atypia.
- Pap IIID: dysplasia (sub-classified into mild, moderate and severe).
- Pap IVa: carcinoma in situ.
- Pap IVb: only one definite tumor cell (carcinoma highly possible).
- Pap V: a lot of definite tumor cells (certain carcinoma).

In cytological evaluation, PapI - IIID<sub>L</sub> (from normal up to mild dysplasia) were considered negative and Pap IIID<sub>M</sub> - PapV were evaluated as positive.

### **2.9.4. Automated image cytometry classification:**

In our analysis, we used the most important two parameters, the 2cDI along with the virtual diagnosis of morphologically abnormal nuclei. Following the recommendations of the ESACP for quantitative cytometry (12), we used the following categories (0, I, II and III) as shown in *table (2)*:

<b>Table 2. Classification of Preneoplasia Stages by AIC</b>		
<b>Grade</b>	<b>Meaning</b>	<b>Description</b>
<b>0</b>	Non-representative	Too few diagnostic cells from the airways
<b>I</b>	Benign	Normal nuclear structures, euploidy, 2cDeviation Index (2cDI) <0.20
<b>II</b>	Suspicious	Samples with more than 1-2% suspicious nuclei, 2cDI >0.20
<b>III</b>	Highly suspicious	2cDI > 0.20, numerous abnormal nuclei, including those with a DNA index of more than five times (5c) the normal haploid DNA content (5cER> 0.2%).

The AIC evaluation was converted from 3 point scale into 2 point scale: 2cDI < 0.2 and objective diagnosis as non-suspicious were all

labeled negative and  $2cDI > 0.2$  and objective diagnosis as suspicious were considered positive.

### **2.10. Follow up of diagnosed preneoplasias:**

All cases of moderate dysplasia or worse were followed up for at least 6 months in 3 months intervals with AIC and AF. These patients were either treated interventionally or conservatively according to the following scheme:

#### **a) Cases of CIS:**

All CIS and worse cases were treated. The choice of treatment modality depended mainly upon the general condition of the patient, his pulmonary function reserve and the multi-centricity of the early tumor. Patients with depleted pulmonary reserves were treated by endobronchial ND-YAG laser. In the meanwhile, surgical resection of the affected segment or lobe was preferred in localized lesions with acceptable preoperative assessment.

#### **b) Cases of moderate-severe dysplasia:**

Cases of moderate to severe dysplasia were given steroid inhalation for 3 months to dampen inflammation and the patient was instructed to stop smoking. If dysplasia persist, treatment and follow up was conducted as previously mentioned in CIS. Usually endobronchial intervention by ND-YAG laser was sufficient.

### **2.11. Methods of statistical analysis:**

Sensitivity and specificity of AIC and/or WLB/AF were tested against the histopathological and /or cytological diagnosis as the gold standard. Statistically unbiased estimates of sensitivity and specificity were not possible to obtain because serial sections of the entire tracheobronchial tree would need to be examined after bronchoscopic procedures for these to be defined. However, since the objective of the study was to determine whether the addition of AF to WLB was better than WLB alone, and whether the addition of AIC to WLB/AF examination would improve the sensitivity of detecting new early lung cancer, the relative sensitivity between WLB/AF to WLB alone was calculated along with the 95% confidence interval (from Ciba Geigy Scientific tables 1980) to detect the contribution of such relatively new technologies in detecting early lung

cancer. Also, the positive predictive value, negative predictive value, diagnostic efficiency were all calculated to evaluate the performance.

The data were evaluated on a per-patient bases and not on a per lesion basis as the latter method has its consequences on further management of individual lesions and not on the rate of detecting new early lung cancer cases.

**Definition of Sensitivity:** The probability of a test to be positive if the disease is truly present.

$$\text{Sensitivity} = \text{True positive} / \text{True positive} + \text{False negative}$$

**Definition of Specificity:** The probability of a test to be negative if the disease is truly absent.

$$\text{Specificity} = \text{True negative} / \text{True negative} + \text{False positive}$$

**Relative sensitivity** = Sensitivity of WLB+AF/ Sensitivity of WLB alone.

A relative sensitivity more than one would indicate a real improvement in detection rate of WLB+AF vs that of WLB alone.

**Definition of Positive Predictive value (PPV):** is the probability of the person of having the disease when the test is positive.

$$\text{Positive Predictive value} = \text{True positive} / \text{True positive} + \text{False positive}$$

**Definition of Negative Predictive value (NPV):** is the probability of the person not having the disease when the test is negative.

$$\text{Negative Predictive value (NPV)} = \text{True negative} / \text{True negative} + \text{False negative}$$

**Diagnostic efficiency:** is the relation between all true examination results and the total patients' population results.

$$\text{Diagnostic efficiency} = \text{True positive} + \text{True negative} / \text{Total number of examinations}$$

$$\text{False positive rate} = \text{False positive} / \text{False positive} + \text{True negative}$$

$$\text{False negative rate} = \text{False negative} / \text{False negative} + \text{True positive}$$

### 3. Results

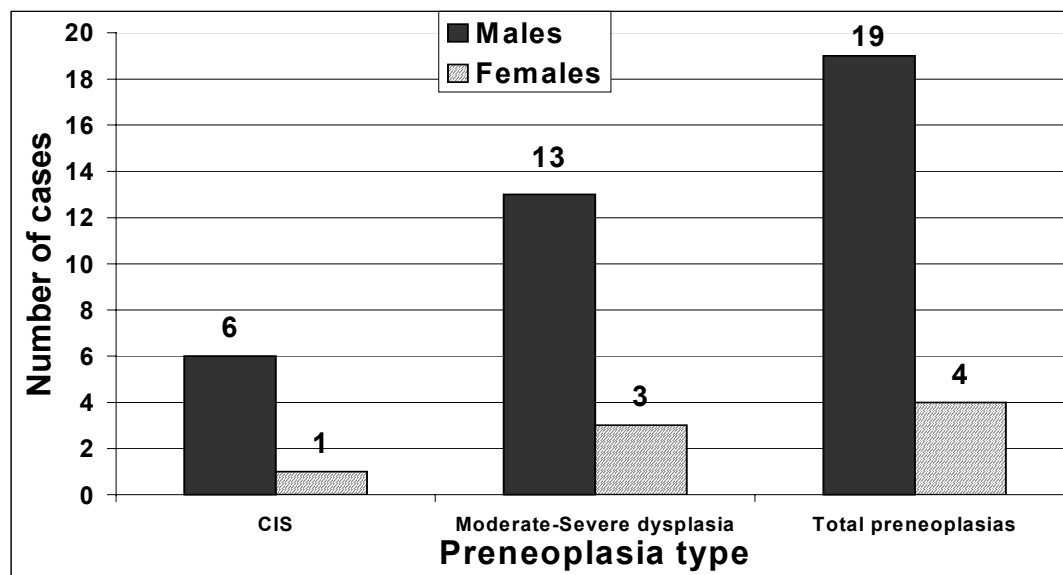
#### 3.1. Analysis of Patients' Characteristics

150 examinations were performed in a total of 119 patients (outpatients as well as inpatients) from January 1999 till June 2000. The population comprised 88 males (74%) and 31 females (26%). Mean age  $\pm$  SD, age range, number of examinations as well as rate of examination per patient are shown in *Table 3*.

**Table 3. Gender, Age Distribution and Examinations per Patient**

	N	Age Mean $\pm$ SD (Years)	Age range	Number of examinations	Examinations/patient
<b>Male</b>	88	62 $\pm$ 10	36-83	115	1.30
<b>Female</b>	31	61 $\pm$ 9.5	42-83	35	1.12
<b>Total</b>	119	62 $\pm$ 9.8	36-83	150	1.26

A total of 23 preneoplasias were diagnosed; 19 cases in males (17%) and 4 cases in females (9%). CIS were 6/19 cases in males (31%) and 1/4 cases in females (25%), while moderate to severe dysplasia were 13/19 cases in males (68%) and 3/4 in females (75%) (*Fig. 11*).



**Fig.11. Types of preneoplasia in relation to gender**



In 88 examinations for smoking males, the mean cigarette consumption was  $42.7 \pm 29.8$  pack years (P/Y) while it was  $30.2 \pm 12.9$  P/Y in 20 examinations for smoking females with a total of 17 cases of preneoplasia occurred among the "smokers" group. Among the 42 non-smokers of both sexes with other risk factors, there were 6 cases of preneoplasia. There was no statistical difference between both groups regarding early lung cancer cases ( $P < 0.48$ ) **Table 4.**

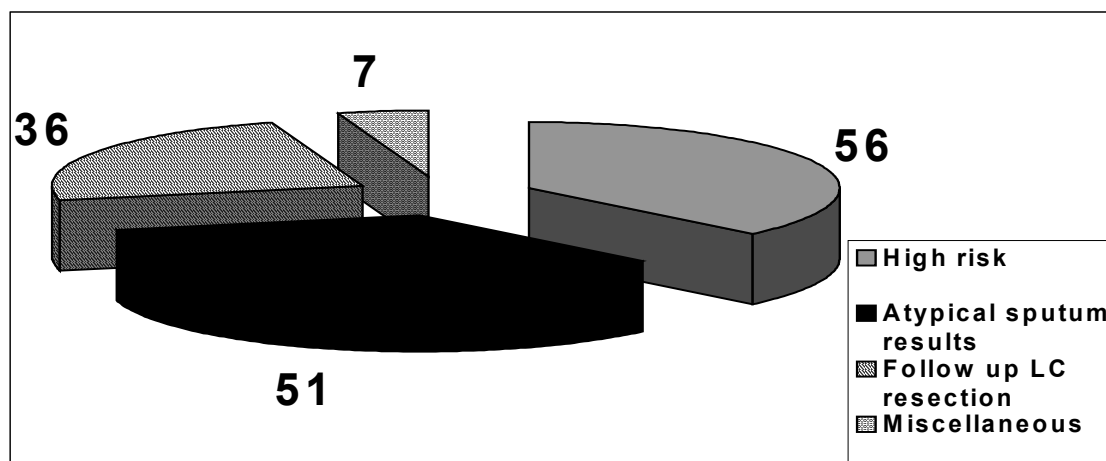
<b>Table 4. Mean cigarette consumption in P/Y and its relation to diagnosed preneoplasias</b>			
	<b>Non-smokers</b>	<b>Smokers</b>	<b>Average smoking for examined cases (P/Y)</b>
<b>Males</b>	27	88	$42.7 \pm 29.8$
<b>Females</b>	15	20	$30.2 \pm 12.9$
<b>Total</b>	42	108	$40.4 \pm 27.9$
<b>Preneoplasias</b>	6/42	17/108	

Many patients had repeated examinations mainly in the follow up of previous atypical histopathology/cytology results or for follow up of resected for cure lung cancer. The majority of patients (82%) had one examination only while a minority had 3 (2%) or 4 (3%) examinations (**Table 5**).

<b>Table 5. Frequency of Bronchoscopic Examinations/ Patient</b>		
<b>Number of examinations/Patient</b>	<b>Number of patients</b>	<b>Final number of examinations</b>
1	98/119 (82%)	98
2	15/119 (13%)	30
3	2/119 (2%)	6
4	4/119 (3%)	16
Total	119	150

A total of 56 examinations (38%) were carried out for high-risk patients for LC presenting with  $\pm$  bronchial symptoms i.e. heavy smokers with positive family history of lung cancer, occupationally exposed workers such as uranium miners, with a change in symptoms like recurrent unexplained hemoptysis or patients with COPD. The group with previous sputum atypia bronchoscope to search for their occult LC lesions was the second largest group comprising 51 patients (34%). 36 bronchoscopies (24%) were done as follow up of resected for cure LC.

Among the group miscellaneous, 7 patients (4%) were recruited with upper respiratory or digestive tract malignancies, implying a higher risk for developing early lung cancer (*Fig 12*).



**Fig. 12. Number of examinations performed for different indications**

The highest percentage of preneoplasia was detected in the group with previous atypia in bronchial secretion (23%). In the other groups, the rate was nearly similar ranging from 14% for miscellaneous group to 11% for each group of patients in follow up of resected for cure LC as well as patients with high-risk for LC. (*Table 6*).

<b>Table 6. Distribution of Positive Cases of Preneoplasia per Indication</b>	
<b>Indication for AF examination</b>	<b>Number of positive cases</b>
1. Previous atypical sputum results	12/51 (23%)
2. High risk, clinically suspected patients for developing LC	6/56 (11%)
3. Follow up of resected for cure LC	4/36 (11%)
4. Miscellaneous	1/7 (14%)
Total	23/150 (15%)

### **3.2. Analysis of AIC Results:**

Whereas representative sputum specimens were obtained in only 87% (130/150) of the patients included for AIC examination, all bronchial washings were satisfactory for AIC assessment. On evaluating the 2cDI as a dependable measure of DNA aneuploidy of airway secretions, the

mean value for truly positive cases were  $0.171 \pm 0.05$  in sputum samples and  $0.183 \pm 0.05$  in bronchial washings. On the other hand, the mean values for truly negative cases were  $0.138 \pm 0.039$  for sputum and  $0.138 \pm 0.037$  for washings representing highly significant difference between truly positive and truly negative cases with  $P < 0.0005$  *Table (7)*.

**Table 7. Evaluation of AIC Results**

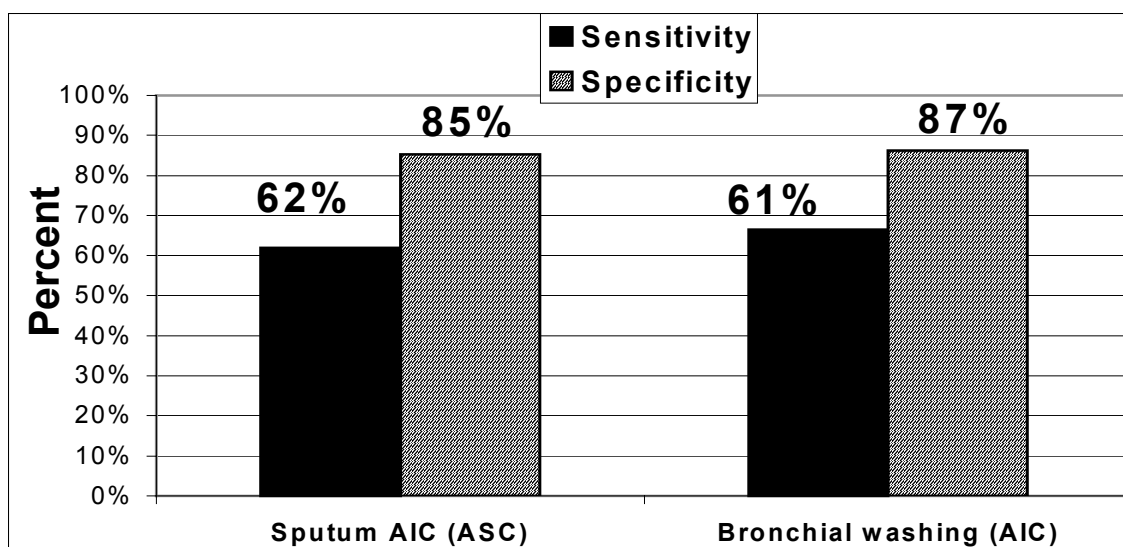
	Number of representative samples	2cDI (mean $\pm$ SD) true positive	2cDI (mean $\pm$ SD) true negative	P-value
<b>Sputum</b>	130/150 (87%)	$0.171 \pm 0.05$	$0.138 \pm 0.039$	$P < 0.0005$
<b>Br. Wash</b>	150/150 (100%)	$0.183 \pm 0.05$	$0.138 \pm 0.037$	$P < 0.0005$

In 5 CIS cases of 7 (71%), a suspicious sample was correctly identified by AIC with a mean 2cDI of  $0.21 \pm 0.055$  for all CIS cases while AIC was only suspicious in 9 cases of moderate to severe dysplasia out of 16 (56%) with a mean 2cDI of  $0.16 \pm 0.056$  for all dysplasia cases showing a highly significant difference ( $P < 0.0005$ ) between 2cDI means in diagnosing both types of preneoplasias (*Table 8*).

**Table 8. Correlation of Cytometric Diagnosis with Histopathological and/or Cytological Diagnosis**

	CIS	Moderate-Severe Dysplasia	
<b>AIC Suspicious</b>	5/7 (71%)	9/16 (56%)	14/23
<b>AIC Non-suspicious</b>	2/7 (29%)	7/16 (44%)	9/23
<b>Total</b>	7	16	23
<b>Mean 2cDI <math>\pm</math> SD</b>	$0.21 \pm 0.055$	$0.16 \pm 0.056$	$P < 0.0005$

Excluding the 20 non-representative sputum samples for AIC examination that were representative in bronchial washings sampling, the sensitivity of both methods as well as the specificity in 130 cases showed no significant difference with a  $P < 0.5$  for both (*Fig. 13*).



**Fig. 13. Sensitivity and specificity of AIC in sputum vs bronchial washings**

### **3.3. Analysis of Endoscopic Results:**

Whenever a suspicious lesion in WLB and/or AF mode was detected, biopsies were taken for histopathological examination. In 90 examinations out of 150, suspicious lesions could not be detected so biopsies were not taken. In 50 examinations (33%), only one biopsy was taken, 2 biopsies in 7 examinations (4%) and 3 biopsies in 3 examinations (2%). A total of 73 representative biopsies were taken and evaluated histopathologically with an overall rate of 0.48 biopsy/examination. In one patient that had two suspicious lesions biopsied, there were two preneoplastic lesions diagnosed in two different lobes which would be reflected on further management measures but not on the rate of detecting early LC cases. The analysis was based on a per patient analysis rather than on a per lesion analysis for lack of consequences (*Table 9*).

<b>Table 9. Biopsy Rate per Examination</b>		
<b>Biopsies /bronchoscopic examination</b>	<b>Number of examinations</b>	<b>Number of biopsies taken</b>
0	90	0
1	50	50
2	7	14
3	3	9
Total	150	73

Biopsy rate is a good indicator for frequency of finding suspicious lesions. It was highest in patients with previous atypical sputum results undergoing meticulous search for the responsible lesion with a rate of 0.78 biopsy/bronchoscopic examination followed by the miscellaneous group at a rate of 0.42 biopsy/examination. In the high risk group of patients, the rate fell to 0.35 biopsy/examination and further in the patients of resected for cure lung cancer (0.27 biopsy/examination) (*Table 10*).

<b>Table 10. Biopsy Rate per Indication</b>		
<b>Indication for AF examination</b>	<b>Number of biopsies per indication</b>	<b>Biopsy rate</b>
1. Previous atypical sputum results	40/51	0.78
2. High risk, clinically suspected patients for developing LC	20/56	0.35
3. Follow up of resected for cure LC	10/36	0.27
4. Miscellaneous	3/7	0.42
Total	73/150	0.48

The final histocytological results of different preinvasive lesions were compared to the suggested image classification under WLB and or AF. Identifying moderate to severe dysplastic lesions, a significantly higher sensitivity for WLB+AF (88%) than WLB alone (44%) was found ( $P < 0.01$ ). On the other hand, there was no significant difference identifying CIS between both modes with a sensitivity of 86% for WLB+AF to 57% only for WLB alone ( $P < 0.27$ ) (*Table 11*).

<b>Table 11. Image Classification in different Preneoplasias</b>			
<b>Suspicious image in</b>	<b>Moderate-Severe dysplasia</b>	<b>CIS</b>	<b>Total</b>
WLB alone not AF	0/16 (0%)	1/7 (14%)	1
AF alone not WLB	7/16 (44%)	2/7 (29%)	9
WLB+AF together	7/16 (44%)	3/7 (43%)	10
None of them	2/16 (12%)	1/7 (14%)	3
Total WLB suspicious	7/16 (44%)	4/7 (57%)	11/23
Total WLB+AF suspicious	14/16 (88%)	6/7 (86%)	20/23
Total preneoplasias	16	7	23

### 3.4. Evaluation of all methods combined:

Of 24 lesions diagnosed in 23 patients as preneoplasia on histopathological and/or cytological basis, 10 were found to be moderate dysplasia, 7 severe dysplasia and 7 carcinoma in situ. In one patient, carcinoma in situ as well as severe dysplasia were diagnosed in two different sites which would influence measures of further management but not the rate of preneoplasia detection. Combining AIC, WLB and AF, there is a sensitivity of 100%. 3 cytologically diagnosed preneoplastic lesions could not be localized. The data of These three patients, marked by (\*) were not completed because of non-compliance (*Table 12*).

<b>Table 12. Characteristics, Localization, AIC and bronchoscopic data of Patients with Preinvasive Lesions</b>							
<b>Patient No.</b>	<b>Age (ys)</b>	<b>Gender</b>	<b>Site</b>	<b>Histo/Cytol</b>	<b>ASC</b>	<b>WLB</b>	<b>AF</b>
1	75	M	LB6	SD	-ve	+ve	+ve
2	73	F	MK	SD	-ve	+ve	+ve
3	65	M	Trachea	MD	+ve	-ve	+ve
4	70	M	RB1-2	MD	+ve	+ve	+ve
5	79	M	RB6	MD	+ve	-ve	+ve
6	63	M	LB6	MD	+ve	+ve	+ve
7	48	F	MLK	MD	-ve	+ve	+ve
8	68	F	LB6	MD	-ve	+ve	+ve
9	66	M	Stump	SD	+ve	-ve	+ve
10	70	M	LB7	CIS	-ve	+ve	+ve
11	64	M	RULB	CIS	+ve	+ve	-ve
12	59	M	LB3	SD	-ve	-ve	+ve
13	46	M	RB6	CIS	+ve	-ve	+ve
14	67	F	LB3	CIS	+ve	+ve	+ve
15	76	M	RB1	MD	-ve	+ve	+ve
16	46	M	RB4-5	MD	-ve	-ve	+ve
17	69	M	LB6	MD	+ve	+ve	+ve
18	57	M	LUL	SD	-ve	+ve	+ve
19	70	M	RB8	CIS	+ve	-ve	+ve
20	57	M	RUL, LB3	CIS SD	-ve -ve	+ve -ve	+ve +ve
21*	60	M	-ve	MD	+ve	-ve	-ve
22*	61	M	-ve	CIS	+ve	-ve	-ve
23*	56	M	-ve	SD	+ve	-ve	-ve

**MD**= Moderate dysplasia; **SD**= Severe dysplasia; **CIS**= Carcinoma in Situ; **M**= Male; **F**= Female; **+ve** = Positive; **-ve** = Negative

Per patient analysis of the final results revealed sensitivity of WLB alone to be 48 % (11/23) while by adding AF, the sensitivity improved up to 87% (20/23). A distinguished finding was the 100% sensitivity reached by adding AIC to both WLB+AF while specificity decreased from 61% for WLB+AF to 55% for WLB+AF+AIC all together. The positive predictive value was the highest for AIC (45%) but showed no difference between WLB alone (33%), WLB+AF (28%) and WLB+AF+AIC (28%) ( $P<0.39$ ). The negative predictive value ranged from a highest value of 100% with WLB/AF/AIC together to a lowest value of 89% with WLB alone (*Table 13*).

**Table (13) Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of all Methods separately and combined**

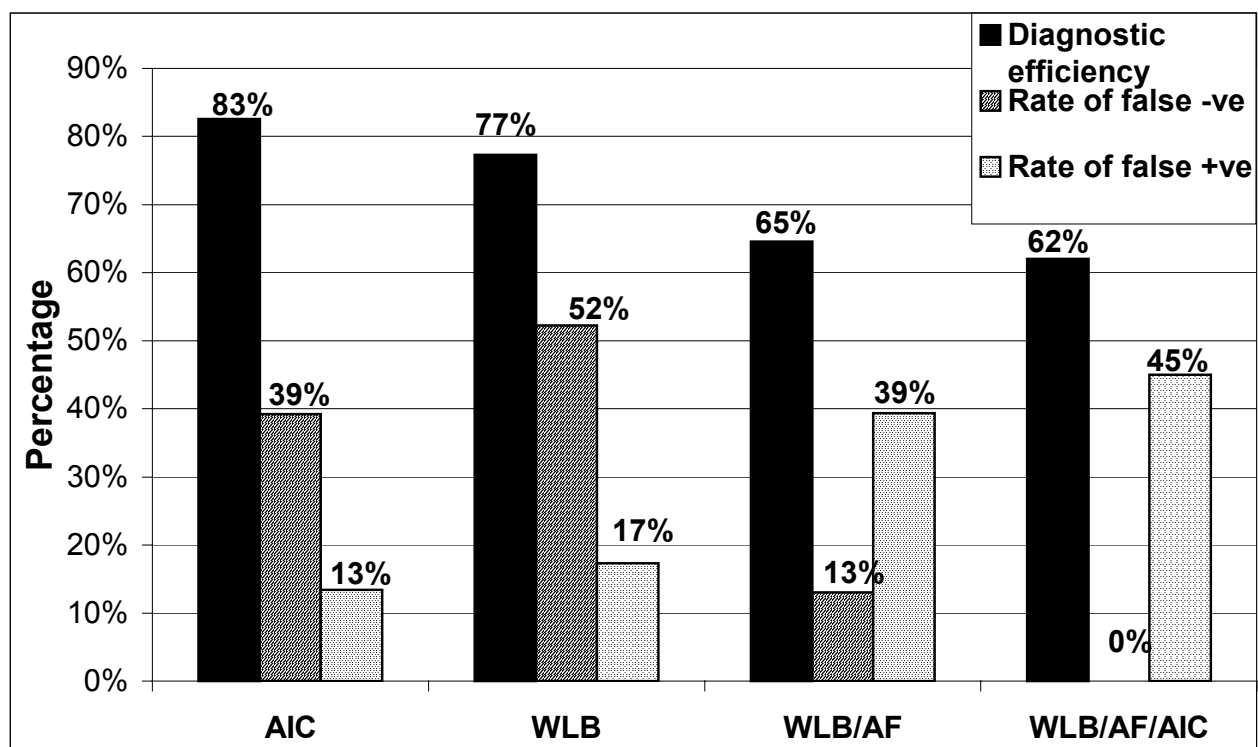
	<b>AIC Br. Wash</b>	<b>WLB</b>	<b>WLB/AF</b>	<b>WLB/AF/ AIC</b>
<b>Sensitivity</b>	61% (14/23)	48% (11/23)	87% (20/23)	100% (23/23)
<b>Specificity</b>	87% (110/127)	83% (105/127)	61% (77/127)	55% (70/127)
<b>*PPV</b>	45%	33%	28%	28%
<b>**NPV</b>	92%	89%	96%	100%

**PPV**, Positive predictive value; **NPV**, Negative predictive value

The improvement in the relative sensitivity of WLB+AF vs WLB alone was 1.8 (95% confidence interval 1.4 - 2.47). Also, the improvement in relative sensitivity of WLB+AF+AIC methods used was significantly better in relation to AIC alone (1.64) but with minimal improvement in relation to WLB/AF (1.15). As regards the relative specificity, there was always decreased specificity for combined methods (*Table 14*).

<b>Table (14) Relative Sensitivity and 95% Confidence Interval (95% CI)</b>			
	<b>(WLB+AF)/ (WLB alone)</b>	<b>(WLB+AF+AIC)/ (WLB+AF)</b>	<b>(WLB+AF+AIC)/ (AIC alone)</b>
<b>Relative Sensitivity</b>	1.8	1.15	1.64
<b>95% CI Sensitivity</b>	1.4 - 2.47	1.02 - 1.28	1.24 - 2.21
<b>Relative Specificity</b>	0.73	0.90	0.63
<b>95% CI Specificity</b>	0.58 - 0.92	0.89 - 0.92	0.50 - 0.81

Final evaluation showed a diagnostic efficiency of 83% for AIC, decreased to 77% with WLB alone, 65% for WLB combined with AF and 62% for all methods combined. The false negative rate was highest with WLB alone and improved up to 0% false negative detection on evaluating WLB+AF+AIC together with a relatively high false positive rate of 45% (Fig. 14).



**Fig. 14. Diagnostic efficacy, false positive and false negative rates**



### **3.5. Follow up results:**

- a) **Patients with CIS:** Of 7 cases diagnosed as CIS, there were 3 cases with complete remission in a 6 months period, one case progressed to invasive cancer and 3 non-compliant patients with no available follow up data. Of the 3 patients in full remission, 2 were treated with ND-YAG Laser alone. One of the CIS cases persisted after laser, full remission was acquired after brachytherapy. The patient who developed invasive cancer was functionally inoperable and progression occurred in spite of ND-YAG laser therapy (*Table 13*).
- b) **Patients with moderate-severe dysplasia:** Of 16 cases diagnosed as moderate-severe dysplasia, 9 cases had a complete remission in 6 months period on steroid inhalation, a case of moderate dysplasia presented after 8 months failed follow up with invasive cancer and 6 non-compliant cases with no available follow up data (*Table 15*).

Summarized there are 12 cases of full remission out of 14 compliant patients (86%) on the mentioned scheme for early lung cancer management while there were only 2 cases (14%) that progressed to invasive cancer.

**Table 15. Results of Follow up for Diagnosed  
Preneoplasias**

<b>Patient</b>	<b>Age (ys)</b>	<b>M/ F</b>	<b>Site of Lesion</b>	<b>Histo/ Cytol</b>	<b>Therapy</b>	<b>Outcome</b>
<b>1</b>	75	M	LB6	SD	Steroid Inhalation.	Full remission
<b>2</b>	73	F	MK	SD	Steroid Inhalation	Full remission
<b>3</b>	65	M	Trachea	MD	Steroid Inhalation	Full remission
<b>4</b>	70	M	RB1-2	MD	Steroid Inhalation	Full remission
<b>5</b>	79	M	RB6	MD	Steroid Inhalation + NC	Invasive cancer
<b>6</b>	63	M	LB6	MD	Steroid Inhalation	Full remission
<b>7</b>	48	F	MLK	MD	Steroid Inhalation	NC
<b>8</b>	68	F	LB6	MD	Steroid Inhalation	NC
<b>9</b>	66	M	Stump	SD	Steroid Inhalation	Full remission
<b>10</b>	70	M	LB7	CIS	EB Laser	Full remission
<b>11</b>	64	M	RULB	CIS	EB Laser	Invasive cancer
<b>12</b>	59	M	LB3	SD	Steroid Inhalation	Full remission
<b>13</b>	46	M	RB6	CIS	EB Laser	NC
<b>14</b>	67	F	LB3	CIS	EB Laser + Brachytherapy	Full remission
<b>15</b>	76	M	RB1	MD	Steroid Inhalation	NC
<b>16</b>	46	M	RB4-5	MD	Steroid Inhalation	Full remission
<b>17</b>	69	M	LB6	MD	Steroid Inhalation	NC
<b>18</b>	57	M	LUL	SD	Steroid Inhalation	Full remission
<b>19</b>	70	M	RB8	CIS	EB Laser	NC
<b>20</b>	57	M	RUL, LB3	CIS SD	EB Laser & Steroid Inhalation	Full remission Both
<b>21*</b>	60	M	-ve	MD	Steroid Inhalation	NC
<b>22*</b>	61	M	-ve	CIS	EB Laser	NC
<b>23*</b>	56	M	-ve	SD	Steroid Inhalation	NC

**MD**= Moderate dysplasia; **SD**= Severe dysplasia; **CIS**= Carcinoma in Situ; **M**= Male; **F**= Female; **NC**= Non-Compliant; **EB Laser** = Endobronchial Laser Therapy

# **Discussion**

## **4.1. Size of the problem:**

The incidence of lung cancer worldwide is often described as reaching epidemic proportions despite the fact that for 90% of these cancers, the cause is known and preventable—cigarette smoking (23). Approximately 10% of heavy smokers will develop lung cancer in the long run (16). Even if all tobacco use were immediately stopped, there would still be many thousands of new lung cancers over the next ten to twenty years, as the risk of contracting the disease doesn't drop on smoking cessation to the non-smokers' level (79).

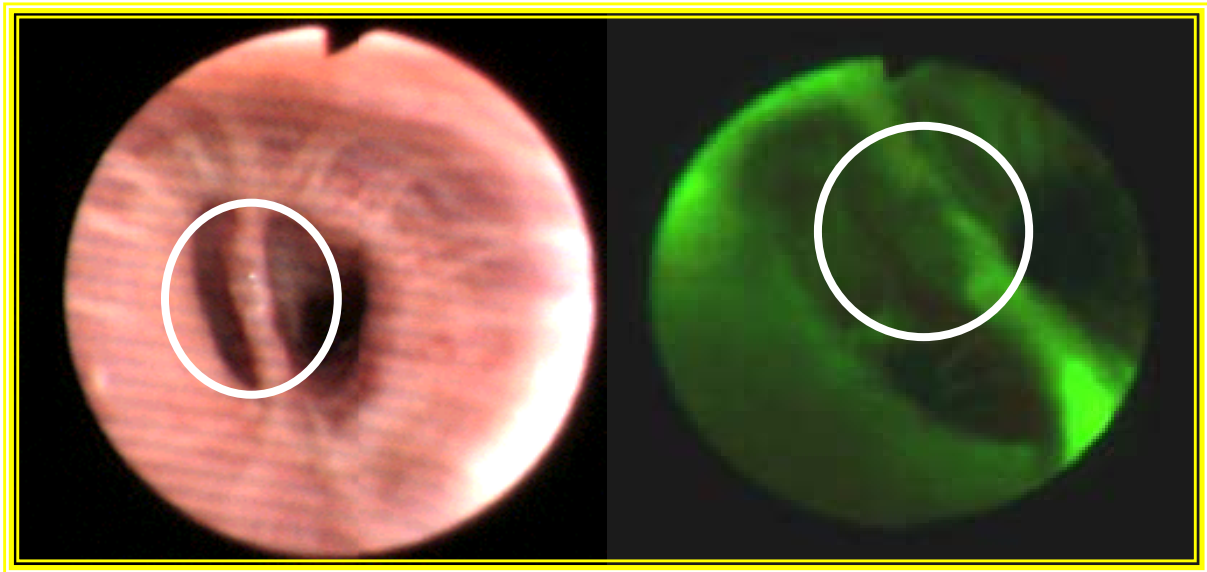
There is no doubt that the earlier lung cancer is detected, the better are the patient's chances of survival (57). *Naruke* et al. in 1997 reported a 100% survival rate when lung cancer was diagnosed in stage 0 while it was only 68.5% for stage I and continued to decrease to 46.9% for stage II, 26.1% for stage IIIA, 11.2% for stage IV and down to 9% for the poorest prognosis stage IIIB (60). So, there is a clear inverse proportion between cancer stage at the time of diagnosis and the survival rate.

Intraepithelial (preinvasive) neoplasia starts with a molecular phase, in which the epithelium is morphologically normal but is undergoing genomic instability, followed by a morphologic phase, in which aberrant proliferative foci with nuclear and cytologic changes, termed dysplasia, and carcinoma in situ form the basis for the microscopic diagnosis of preinvasive or intraepithelial neoplasia (14).

Despite intensive research, /clinical screening trials, and enormous therapeutic costs, there has been little improvement in survival in the last 30 years. The ineffectiveness of current treatments and the observation that early stage disease has the best outcome has led to a belief that the early detection of the disease offers the best chance of survival.

## **4.2. Inefficiency of conventional methods:**

Carcinoma *in situ* and microinvasive cancers of the central bronchi present a challenging diagnostic problem. These cancers mostly are first a few cell layers thick (0.2-1mm) and a few millimeter in surface diameter (85). Because of this, the lesion may not be recognized by conventional methods previously tried to early diagnose LC (**Fig 15**).



**Fig. 15 CIS in carina of medial segment of Rt. lower lobe**

For decades, the diagnosis of early lung cancer depended upon conventional methods of low sensitivity. Chest x ray, as expected, is unable to detect early central LC lesions in its intra-epithelial stages. Its use alone as a method of screening for this type of cancer will cause a 3 years delay in its diagnosis if not combined with sputum cytology (55). Sputum cytology was found to be of low sensitivity (40%) in detecting occult stage I LC in the screening studies conducted by Johns Hopkins Lung Project, Mayo Lung Project and the Memorial Sloan-Kettering National Lung Program 20 years ago. Also, regarding conventional white light bronchoscopy, detection and localization of dysplasia and carcinoma in situ was found not to exceed 29% (84).

#### **4.3. Failure of early screening studies:**

Based on the fact that “the earlier lung cancer is detected, the better its prognosis”, large screening trials were conducted in the 70's using various combinations of sputum cytology, CXR and conventional bronchoscopy, in an effort to increase survival in high-risk patients. For a number of reasons, including antiquated technologies, these trials failed and health authorities turned to anti-smoking measures as the only viable alternative to combat lung cancer. But as the evidence mentioned above shows: even if anti-smoking strategy were strictly followed, lung cancer will continue its steep rise for another two decades before it will finally levels off and start to drop.

To summarize, it was found that the value of chest x-rays in screening is debatable, no added advantage for using sputum cytology could be proved and both methods did not effectively influence the mortality rate of lung cancer. Screening for lung cancer was abandoned as a waste of resources (4, 27).

Meanwhile, new diagnostic methods, discussed here, have re-awakened hopes for effective early LC screening. Hence a two pronged strategy against lung cancer may now be implemented: on one hand, redoubling efforts to reduce smoking in adults and preventing the youth from starting the habit. On the other hand, instituting studies using new promising sensitive technologies for early lung cancer detection. In December 1998, an international conference for prevention and early diagnosis of lung cancer was held in *Varese*, Italy that was attended by 18 world-wide distinguished scientists in this field from eleven countries. A conclusion statement that was agreed unanimously stressed upon the risk of this global lung cancer epidemic and its deadly potential, the pressing need for effective screening measures and the favorable outcome of case finding studies when lung cancer is early detected (34).

#### **4.4. Discussion of results:**

In a case finding study involving 119 patients - performed in 150 examinations- at high risk for LC, we tested the hypothesis, whether by automated image analysis of sputum and bronchial washings and by the addition of AF to conventional bronchoscopy, a significant increase in the diagnosis of severe dysplasia and CIS could be achieved.

As previously noted, patients for this study were recruited with a high risk factor for lung cancer. Patients were either heavy smokers, occupationally exposed as uranium miners, having COPD, sputum atypia, undergoing follow up of previously resected for cure lung cancer or with upper aerodigestive tract cancer (*Fig.12*). These risk factors may be responsible for the high preneoplasia rate among non-smokers (*Table 4.*). This concurs with the recommendations, recently published by *Kennedy* and coworkers in 2000 at SPORE in University of Colorado, recommending narrowing of case finding studies to such higher risk group of patients with smoking history >30 P/Y and COPD. They hope to identify, through these measures, a suitable target population for future screening programs (41).

Based on histopathology and/or cytology, 23 preneoplasias (16 cases of moderate to severe dysplasia and 7 carcinomata in situ) were diagnosed. A relative sensitivity of WLB/AF to WLB alone proved to be 1.8. The sensitivity of combined WLB/AF/AIC proved to be 100% (23/23) for detecting all preneoplasia cases. Although the use of AF, the preneoplastic lesions were not localized in 3 cytologically diagnosed cases out of 23 (**Table 12**). The highest percentage of preneoplasia was detected in the group with previous atypical bronchial secretion results (23%) which was the same finding in a previously published study by *Khanavkar* et al. 1998 (42). This group had the highest rate of biopsy taking per examination (0.78) in the current study, which reflects the frequency of finding suspicious lesions and consequently explains this high detection rate (**Table 10**). After excluding 9 non-compliant patients from follow up analysis, a success rate of 12/14 cases (86%) in treating preneoplasia was achieved.

In this series, 150-sputum specimens as well as bronchial washings were collected for AIC examination. Sputum samples were collected without induction aiming to evaluate the detection rate by a simple technique that may be applicable for future screening programs. In 13% of cases (20 patients), sputum was found to contain a minimal amount of respiratory tract cells and was classified as non-representative for evaluation while all bronchial washings were representative (**Table 7**). This may emphasize the importance of sputum induction and its value in such circumstances. Such simple technique should be routinely used in any future sputum-examination studies. After excluding the 20 non-representative cases from evaluation, the sensitivity of bronchial washings vs sputum for AIC were 61% and 62% respectively showing no statistically significant difference ( $P < 0.5$ ). Similarly, no difference was observed in specificity of bronchial washings (87%) and sputum specimens (85%) ( $P < 0.5$ ) (**Fig. 13**). The overall diagnostic efficacy for AIC in detecting preneoplastic lesions of the lung was 83% (**Fig. 14**).

Many investigators have shown that the more advanced the malignancy grade of a certain tumor, the higher and more widely dispersed is the DNA content of the tumor cell nuclei (43, 63). These two changes raise the mean square deviation of the nuclear DNA content from the diploid and thereby increase the 2cDI (13).

On evaluating 2cDI as a measure for DNA aneuploidy, a highly significant difference ( $P < 0.0005$ ) could be found between truly positive and truly negative cases. The mean 2cDI for true positive cases by bronchial washings was  $0.183 \pm 0.05$ . The other parameter we used for

evaluating suspicious nuclei was the virtual morphological diagnosis of suspicious nuclei, which didn't usually correlate with the 2cDI value in this group of patients. Further research will have to go into trying to redefine threshold values for 2cDI in preneoplasia that is different from values for invasive cancer (*Table 7*).

By evaluating the different preneoplastic stages separately, it was found that AIC correctly identified 71% of CIS cases with a mean 2cDI of  $0.21 \pm 0.055$  while the detection rate for dysplasia was 56% with a mean 2cDI of  $0.16 \pm 0.056$  showing a highly significant difference in detection rate between both types of lesions ( $P < 0.0005$ ). This supports the observation that more advanced lesions present with more pronounced abnormalities in nuclear structure, which will be consequently reflected on cellular proliferation rate and 2cDI (*Table 8*).

In the current study, white light bronchoscopic examination was always followed by autofluorescence bronchoscopy. Some authors who tested sensitivity and specificity of each mode alone have done randomization of the sequence of different modes. They proved no difference in the rate of early detection irrespective of the sequence (80). AF alone will miss some endoscopic information provided by WLB as submucosal tumor growth. AF should be used as a complementary examination to WLB and not exclusively. To assess the additional benefit added by AF, it is appropriate to start examination with WLB and to compare localization rate of WLB alone to WLB+AF.

Histo/cytologically confirmed moderate and severe dysplasias vs CIS results were correlated to their WLB and /or AF image classification and the sensitivity for each preneoplasia type were determined. There was a significant difference between WLB+AF vs WLB alone in detecting moderate/severe dysplasia lesions with a sensitivity of 88% and 44% respectively ( $P < 0.01$ ). On the other hand, there was no statistical difference between both methods in detecting CIS with a sensitivity of 86% for combined modes vs 57% for WLB alone ( $P < 0.27$ ) (*Table 11*).

In 1993, **Lam** et al. calculated the average red and green intensities of autofluorescence images for normal bronchial mucosa and preneoplastic lesions (47). They concluded that there was a significant difference between fluorescence intensities in images of normal and preneoplastic lesion but there was no such difference between dysplasia and CIS. Yet, in partial support to the current study results, **Lam** et. al in 1994 retrospectively correlated different preneoplasia grades to image classification without further statistical interpretation regarding difference

between both modes in detecting preneoplasia grades. After analyzing their data, it was found that they had a highly significant difference between WLB+AF vs WLB alone in detecting moderate/severe dysplasia with a rate of 63/78 and 30/78 respectively ( $P<0.001$ ) (48). In contrast to the results of this study, There was a significant difference regarding CIS detection with a rate of 32/35 for WLB+AF vs 14/35 for WLB alone ( $P<0.001$ ).

To summarize, the current study supports that there is a statistically significant difference between WLB+AF vs WLB alone in detecting moderate to severe dysplasia ( $P<0.01$ ) while such difference was not evident in case of CIS lesions. This finding is comparable with some previous studies. The explanation of this difference is not clear. The inter-observer variability is an important factor. Also, long term use of AF trains the observer's eye for subtle endoscopic changes in WLB as well. Finally, there are more marked endoscopic findings in WLB for CIS than dysplasia.

In this study, the relative sensitivity of WLB+AF vs WLB alone was found to be 1.8 for early lung cancer lesions and preneoplasia with a sensitivity of 87% (20/23) vs 48% (11/23) respectively ( $P<0.005$ ). Thus, the addition of AF to WLB resulted in a significant increase in detection rate of early lung cancer lesions that would otherwise be missed if AF were not used. By adding AIC for bronchial washings, relative sensitivity of WLB+AF+AIC vs WLB+AF was improved by 1.15 with a sensitivity of 100% (23/23) for all methods together. This comes in support of the complementary concept between different methods of early LC detection (*Tables 13,14*).

Three lesions confirmed by cytology could not be localized successfully. This may be because these lesions were possibly located in the subsegmental airways that are accessible to bronchial washings but not to the bronchoscope (silent area of the lung), or they may represent false negative lesions for WLB/AF. This phenomenon is known in other studies in which positive control biopsies were found in non-suspicious endoscopic areas. Follow up data for these three cases could not be obtained because of the patients' non-compliance (*Table 12*).

The positive predictive value for both WLB+AF and WLB+AF+AIC was 28%, which was comparable to that of WLB alone (33%). On the other hand, the negative predictive value is ideal, ranging from 89% for WLB alone to 96% for WLB+AF and up to 100% for WLB+AF+AIC together. Although the low positive predictive value is



not ideal, the increased sensitivity as well as negative predictive value of the fluorescence examination combined with AIC is a true advantage (*Table 13*).

In 1993, **Lam** et al. encouraged the concept of a two-stage screening approach where a simple, highly sensitive, but not necessarily specific, initial test is followed by a more involved, highly specific confirmatory test (50). In the current study, the relative specificity of WLB+AF vs WLB alone was found to be 0.73 for early lung cancer lesions with a specificity of 61% and 83% respectively. Along with that, the relative specificity of WLB+AF+AIC vs WLB+AF was 0.90 with 55% of the former. Thus, the addition of AF to WLB resulted in a significant decrease in specificity from 83% to 61%. In contrast, adding AIC to WLB+AF led to no significant deterioration in specificity than before with 55% vs 61% (*Tables 13,14*).

This decrease in specificity due to false positive results may be attributed to the assumption that the autofluorescence pattern of bronchial mucosa may reflect molecular genetic abnormalities beyond the threshold of the microscopic abilities of the pathologist (46). This is supported by **Venmans** et al. 2000 who described two cases of severe dysplasia and another of carcinoma, which were previously and frequently classified as AF suspicious but biopsies at that location had revealed normal mucosa only. Progression occurred later on in the previously AF suspected sites (81). These observations are considered a rich field for future controlled studies concerning the correlation between AF image classification and molecular genetic abnormalities.

#### **4.5. Comparison with previous international studies:**

There is no available similar study up till now to evaluate the feasibility of combined automated image cytometry and autofluorescence bronchoscopy in improving early lung cancer detection and localization. A comparison between the results of this study for each method separately with any comparable studies available will be commented on.

Since the development of the LIFE<sup>®</sup> device, several studies from international centers have been published on results and evaluation of the LIFE system in early detection of precancerous lesions and CIS. Two large studies were reported from **Lam** et al. in 1993 and 1998 (47, 46). Also, a large prospective study were published by **Khanavkar** et al. in 1998 from the Research Institute for Diagnosis and Treatment of Early Lung Cancer (RIDTELC<sup>™</sup>), in Bochum, Germany (42) and there are

data by *Venmans* et al. from the Free University Hospital of Amsterdam in 1999 (82). *Kurie* et al. from Texas University, Houston reported his results in 1998 (44) as well as *Kato* et al. 1998 (39), *Yokomise* et al. 1997 and *Kakihana* et al. 1999 from Japan (86, 37).

The results of the above mentioned studies regarding number of positive lesions, sensitivity, specificity, relative sensitivity, positive predictive value and negative predictive value are summarized together with the current study's data (*Table 16.*):

<b>Table 16. Comparison of available results from previous autofluorescence studies with the current study</b>						
<b>Author</b>	<b>+ ve lesions/ total</b>	<b>WLB Sens.- Spec</b>	<b>WLB+AF Sens.- Spec</b>	<b>Relative sens.</b>	<b>PPV WLB- W+AF</b>	<b>NPV WLB- W+AF</b>
<b>Lam 1993 (47)</b>	77/328	48.4% - 94%	72.5% - 94%	1.5	-	-
<b>Lam 1998 (46)</b>	142/700	37.3% -?	75% -?	2.0	0.39 -0.33	-
<b>Khanavkar 1998 (42)</b>	38/194	31.8% -75.4%	86.4% -31.4%	2.7	-	-
<b>Venmans 1999 (82)</b>	79/681	59% -85%	85% -60%	1.4	35% -23%	94% -97%
<b>Kurie 1998 (44)</b>	0/245	-	43.3% -57.3%	-	? -24.8%	? -75.7%
<b>Kato 1998 (39)</b>	73/213	51% -59%	93% -65%	1.8	76% -66%	-
<b>Yokomise 1997 (86)</b>	19/51	65% -71%	90% -77.4%	1.4	-	-
<b>Kakihana 1999 (37)</b>	79/147	51% -54%	88% -56%	1.7	62% -71%	-
<b>Current study 2000</b>	23/150	48% -83%	87% -61%	1.8	33% -28%	89% -96%

The results of the current study are comparable with previous international studies to a large extent. There are, however, certain differences in the study design as well as the methods of statistical analysis that need to be considered.

Nearly all the previous studies in this comparison included patients with manifest invasive endobronchial tumors in their study group with the aim of detecting early lung cancer lesions in other sites of the bronchial tree as these patients are at higher risk for harbouring a second primary LC. These pioneer studies aimed mainly at testing the relative sensitivity between WLB+AF to WLB alone in detecting ELC lesions because, at that time, AF itself was still under trial. The AF used was LIFE<sup>®</sup> in all studies except that of *Kakihana* et al. 1999 who used the SAFE 1000 device, which uses a conventional Xenon light with a special filter that delivers only the wavelength of 420-480 nm as an excitation light (37).

In this study, sensitivity and specificity were tested against histopathological and/or cytological diagnosis as the gold standard. Ideally speaking, statistically unbiased estimates of sensitivity and specificity were not possible to obtain because serial sections of the entire tracheobronchial tree would need to be examined after bronchoscopic procedures for these to be defined. In all studies mentioned, control biopsies were taken from different predetermined segments after proving WLB and AF negative. This was done to determine true negative cases and to calculate specificity. Some studies reported no histopathologically positive control biopsies for preneoplasia (80), others detected insignificant number of positive control biopsies (48, 86) while in only one study, no difference was found between biopsies taken from predetermined sites and AF guided (44).

In the current study, control biopsies were not taken but bronchial washings for conventional cytology instead. Control biopsies were not done, as it does not provide the ideal solution for calculating sensitivity and specificity because some sites of bronchial mucosa that escape biopsies may harbour preneoplasia. Also, it can't detect peripheral preneoplastic lesions in the bronchoscopically silent subsegments - a true advantage for conventional cytology. Considering repeated AF follow up examinations of these high-risk patients, biopsy sites will leave permanent AF suspicious area. Even if some control biopsies were found to be preneoplastic, this would not be reflected on the study recommendations, as this couldn't be considered a standard investigation to be followed, as bronchial washings for cytology.

In their statistical analysis, some authors calculated sensitivity and specificity of these systems in detecting moderate dysplasia and worse including invasive lung cancer, which was sometimes in large numbers as 15/19 and 40/142 (86, 46). This will increase the sensitivity of WLB in relation to AF as WLB can be considered to be almost 100% sensitive in diagnosing invasive tumors. On the contrary, others calculated their results for the detection of metaplasia and mild dysplasia, which is actually not an indication for AF use (44).

In the current study, statistical analysis of data was done on a per patient basis only while all other studies calculated their results mostly on a lesion-by-lesion analysis rather than a per patient analysis. A per lesion analysis is ideal for studying of AF sensitivity itself in identifying individual preneoplastic lesions that may be many lesions in a single patient with already diagnosed invasive LC.

Difference in experience between examiners and pathologists may explain the differences in results between studies. In the multi-centric study (eight centers) (46), a relative sensitivity of 6.3 was calculated for moderate to severe dysplasia and CIS lesions between WLB+AF and WLB alone with a detection rate of only 9/102 for WLB in comparison to 55.9% for the combined method in detecting such lesions. This puts a big question mark on the experience of the responsible examiners in these centers regarding the WLB endoscopic diagnosis.

The study of *Kurie* et al. 1998 used a historic control group for WLB to compare it with the study group for AF. In addition, a reference pathologist found 18 dysplastic lesions versus the original eight detected by the study's pathologist in an independent review of the biopsy specimens magnifying the problem of different pathological interpretation between different observers. Finally, 46% of the study population were women, which may explain the low yield of preneoplasia in this study (44).

*Venmans* et al. 1999 randomized the sequence of AF/WLB. He found no difference in the detection rate between starting with either method. Also, he differentiated the results of the first half of examinations from the second half trying to identify the learning effect. He found that results obtained in the second to be better than the first indicating a learning effect in using AF (82). Comparing our results and that published by *Khanavkar* et al. 1998, we realize that there is improvement in all parameters of WLB and AF in the current study, with the exception of AF sensitivity that was nearly the same, indicating a

positive learning curve for AF in detection of subtle intraepithelial preneoplastic lesions (42).

**Kakihana** et al. 1999 conducted his study with SAFE 1000 and reported 79 positive lesions out of 147 with 24 lesions of invasive cancer. He did not classify dysplasia according to severity (37).

Finally **Kato** et al. 1998 published their results in an editorial comment on the method of AF in detecting ELC not describing any detailed analysis or study design. He mentioned that 41 sites with invasive cancer out of 213 total biopsy sites (39).

To summarize, nearly all available studies, including the current one, proved the superiority of combined WLB+AF to WLB alone in detecting preneoplasia of bronchial mucosa. These results were recognized by the US Food and Drug Administration when they approved the LIFE<sup>®</sup> system.

Regarding AIC, a study was conducted on 28 cases exhibiting atypical changes in sputum cytology and one case in bronchial washings. They reported 19 cases out of 28 proved by different diagnostic methods to be bronchial cancer. Of these, 17 cases proved true positive by AIC leaving a sensitivity of 89.5% and a specificity of 100% with a range of 3 days to 6 months between AIC diagnosis and morphological confirmation by conventional methods. They concluded that DNA aneuploidy detected by AIC of sputum may proceed the morphological criteria of malignant transformation discovered by conventional cytology and or histopathology (7).

Another group conducted their study on 142 suspicious lung cancer patients and 50 controls by collecting bronchial washings and correlating the results with the end diagnosis which comprised cytology, histopathology and/or post resection pathological diagnosis. they reported 102 cases with end diagnosis of bronchogenic carcinoma with a sensitivity of 90% and specificity of 84% for AIC. The remarkable difference from our study was that the recruited patients were completely different regarding their inclusion criteria. Such patients with invasive lung cancer would be excluded from the current study, which deals mainly with preneoplasia detection (54).

From the current study, the benefit of AIC was obvious in detecting DNA aneuploidy of early lung cancer nuclei. We recorded a sensitivity of 61% (14/23), a specificity of 87% (110/127), positive and

negative predictive values of 45% and 92% respectively with a total diagnostic efficacy of 83%. The complementary effect between AIC and WLB+AF in detecting early lung cancer lesions resulted in 100% detection of all preneoplastic lesions in the study group. These simple and relatively non-invasive techniques are currently under evaluation to be implemented in screening programs for early lung cancer in high-risk groups.

#### **4.6. Follow up and Management of dysplasia and CIS cases:**

In 7 cases of CIS, three were non-compliant and could not be evaluated. In 3 compliant cases out of 4 (75%), complete remission was achieved after endobronchial laser therapy in two of them and in one case after brachytherapy. In the fourth case (25%), invasive cancer developed in spite of laser treatment (**Table 15**). This comes in support to the short-term follow up study reported in 1997, when 22% (7/32) cases of CIS progressed to invasive cancer in 3 months after diagnosis (76).

In cases of moderate to severe dysplasia, there was only 1 case (moderate dysplasia) out of 16 (6%) that progressed to invasive cancer during 8 months after initial diagnosis. Steroid inhalation and follow up AIC and AF after 3 months were recommended. The patient presented eventually after 8 months with invasive cancer. This demonstrates that dysplastic lesions should be regarded as having a high potential of becoming invasive cancer. *Venmans* et al. 2000 observed a similar pattern when a case of severe dysplasia progressed to invasive cancer 10 months after diagnosis. This observation puts a question mark behind the needed time for evolution of dysplasia to invasive cancer suggesting a more rapid transition (81).

The outcome of other dysplasia cases was 56% (9/16) with complete remission, in 37% (6/16) no follow up data were available. Whether remission occurred spontaneously or in response to steroid inhalation is not clear. In 1982, *Johnston* reported on 43 patients out of 135 (32%) who had "atypical cells suspicious of malignancy" in their sputum analysis, the follow up proved these to be due to a variety of non-neoplastic diseases, usually inflammatory in origin (36). This comes in support to our suggested scheme of prescribing inhaled steroid to these patients in order to exclude such inflammatory etiology for dysplasia.

There is no special scheme internationally agreed upon for follow up of diagnosed preneoplasias. Each center deals with individual cases according to a lot of changeable factors such as the availability of new

technologies in each center, skills of the treating physician in interventional endobronchial methods of early cancer treatment etc.

Taking into consideration that dysplasia of bronchial mucosa is sometimes recorded in association with severe lower respiratory tract infection that is considered the responsible insult for such changes in cellular proliferation, we suggest the following scheme for preneoplasia management:

#### **4.6.1. Management of CIS Cases:**

All CIS and worse cases are already lung cancer according to TNM classification for international staging of lung cancer in 1986 and must be treated. The choice of treatment modality depends mainly upon general condition of the patient, his pulmonary function reserve, the degree of tumor invasion, tumor stage and the multi-centricity of the early tumor. A depleted pulmonary reserve as commonly seen in COPD cases will exclude surgical interference and direct the management into the previously discussed endobronchial treatment modalities as ND-YAG laser, electrocautery, cryotherapy, brachytherapy and photodynamic therapy.

A great advantage of ND-YAG laser in this respect over other modalities is that it works in a non-touch technique, which reduces patient coughing during the procedure, keeps the Laser catheter always clean from debris. These advantages made ND-YAG laser the method of choice in RIDTEC™ institute for treating such lesions.

In the meanwhile, surgical resection of the affected segment or lobe is preferred in localized lesions with acceptable preoperative assessment. Further follow up after either modality of treatment include sputum for AIC and AF examination in intervals of 3 months after treatment, 6 months then once yearly.

#### **4.6.2. Management of dysplasia cases:**

Cases of metaplasia or mild dysplasia are followed up after 3 months by sputum for AIC only without any need for further interference. Cases of moderate to severe dysplasia are given steroid inhalation for 3 months to exclude severe inflammation and the patient was instructed to stop smoking. The follow up by both sputum for AIC and AF is scheduled for 3 and 6 months intervals. There is usually complete remission under this strategy but if dysplasia persists, treatment and follow up should be

conducted as previously mentioned in CIS. Usually endobronchial intervention by ND-YAG laser suffices.

#### **4.7. Implications of AIC and AF on future screening studies:**

The past screening studies available proved that a forward shift of stage distribution is possible, especially in case of squamous cell carcinoma with markedly increased rate of resectability (26). However, there was no disease-specific reduction in mortality: a “gold standard” of efficacy in screening for early fatal disease. Innovative technology advancements in the last few years, including AF and AIC as well as new methods of sputum processing (MARATHON-S) promise a worthwhile retrial of such screening studies.

In this study, the complementary effect of both AIC for bronchial washings and AF mounted to a sensitivity of 100% for early lung cancer detection in this group of patients. The economic factor, the feasibility of such a screening program as well as the detection of high-risk group of patients that gain benefit from the program, are all important factors to be considered. Recently in RIDTELC™, a new screening project for ELC detection was started in patients above 50 Ys with a smoking history of 30 Pack/Year using AIC, Conventional cytology and CXR for detecting new cases as well as AF for localizing suspicious lesions. These techniques were chosen to be evaluated and compare its results so as to determine the best and most feasible combination to achieve an acceptable complementary effect for this task taking into consideration the cost-benefit ratio (59).

Sputum examination for cellular atypia is a very practical and useful method of screening. The type of examination, method of sputum preparation, tumor type as well as tumor stage and location are important factors in determining the results. In early lung cancer cooperative study 1984, conventional sputum cytology proved an overall sensitivity of 40% in detecting occult stage I lung cancer, specificity of 99% for moderate-severe dysplasia and CIS and a specificity of 99.95% for lung cancer in general.

In a previous study, an overall sensitivity of AIC for bronchial washings in lung cancer proved to be 90% with a specificity of 84% (54). In the current study, a sensitivity of AIC for sputum examination, without induction, in moderate-severe dysplasia and CIS proved to be 62% with a specificity of 85%. This improvement in sensitivity of sputum examination for AIC together with full automation of the process



provided an advantage for AIC over conventional cytology in screening for lung cancer.

In 1997, *Payne* et al. retrospectively examined 73 slides stored from the Mayo screening project by AIC. 40 slides were of cases that developed LC after a mean of 14 months and 33 slides of normal controls. They described what is called Malignancy Associated Changes (MACs) discovered in slides of patients who developed LC later on. With a special trainable classifier for MACs, a correct detection rate of 77.5% (31/40) in cancer group and 78.8% (26/33) in non-cancer group were achieved. Interestingly, MACs cells disappeared after successful resection of the tumors- a proof of validity as a follow up of resected for cure LC patients (64).

#### **4.8. Other recent advances in ELC detection:**

Several recent advances in understanding the biology and genetics of the carcinogenic process have renewed hope that an early detection strategy may be useful.

##### **4.8.1. Monoclonal antibodies (MoAb) in Sputum:**

*Tockman* et al. in 1988 suggested that diagnostic lead time might be extended by adding a specialized procedure as an adjunct to sputum cytology (i.e., using two MoAbs directed at a difucosylated Lewis X epitope and a 31-kd protein) (78). Using archived sputum specimens from subjects who developed lung cancer after documented moderate dysplasia during the Johns Hopkins study, the investigators applied this procedure to demonstrate positive staining on smears as much as 24 to 48 months prior to the diagnosis of cancer by Saccomanno cytology criteria. Studies are still underway to prospectively validate this technique.

##### **4.8.2. Genetic Advances:**

An increasing number of important mutations have been identified in lung cancer, including allelic deletions or tumor suppressor gene inactivation in 3p, 5q, 9p, 11q, 17p, 13q, 18q, and 22q. At least three alleles appear important on 3p, with more extensive loss commonly seen in small cell lung cancer and less extensive loss associated with non-small cell lung cancer. P 53 alterations appear to be nearly universal in solid tumors, including lung cancers.

Array chip technology promises to accelerate the process of identifying important mutations and critical patterns of mutations. Thus, elucidating molecular-biological events is key to improving the sensitivity of sputum evaluations and may lead to effective chemoprevention strategies, as well as improved treatment of systemic disease. These methods are, however, still in experimental stages and mostly not enough validated to justify blinded clinical trials (41).

#### **4.8.3. Low dose CT of the chest:**

Improved chest imaging also may provide increased sensitivity and improved specificity for localizing early stage lesions of the peripheral lung. Kaneko et al. in 1996 screened 1,369 subjects with at least 20 pack-year smoking histories using spiral CT chest examinations. They discovered 15 cancers (14 stage I), including 11 not detectable by plain chest radiographs. Spiral CT imaging takes 15 to 30 seconds allowing complete chest imaging in one breath-hold. In addition, it has the radiation exposure of a mammogram and can pinpoint lesions as small as 2 to 3 mm in size (38).

These newer, more sensitive technologies have led some scientists to raise valid concern that over identification of benign lesions may lead to possible over treatment morbidity, similar to that experienced with early CT scans. Care must be used to resolve this issue. *Shimizu* et al. in 1995 have suggested using helical CT imaging to improve anatomic distinctions between nodules, vessels, bronchi, and chest wall over conventional CT imaging; this may reduce the incidence of false-negative malignant diagnoses (73).

Bronchoscopy and positron emission tomography (PET) scanning – the latter, however, at present prohibitively expensive - are quite benign and viable approaches to distinguishing between benign and malignant lesions preoperatively, with transthoracic needle aspiration considered less so because of a significant rate of pneumothoraces. Periods of follow-up evaluation CT scans have been used successfully to reduce inappropriate surgery.

Improved imaging also serves a critical role in addressing the increasing incidence of adenocarcinoma (often arising peripherally), as well as in shrinking the "silent" area of the lung i.e., the subsegmental airways not accessible to the bronchoscope.

#### **4.9. Some ELC detection-related problems:**

There are some inherited problems regarding early lung cancer detection. These are partially related to the diagnostic methods and partially related to the unclear carcinogenesis and progression of preneoplasia.

One of these limitations regarding used methods is the absence of quantitative measurements of abnormally interpreted autofluorescence image of a certain lesion. There are some developed systems that depend upon ratio fluorometry, which is a non-imaging technique that quantitatively measures the fluorescence in the lesions but can't sufficiently localize them (45). In recent imaging AF devices, The interpretation of autofluorescence image depends mainly upon the experience of the examiner and this problem is actually the main reason of inter-observer variability in AF interpretation. Recently, *Lam* et al. 2000 presented the preliminary result of a new quantitative fluorescence imaging device that can alert the endoscopist to suspicious areas by numeric signal and an audio tone. He reported the accuracy of this new system in detecting small pre-invasive lesions and the ability to distinguish between preneoplasia and previously biopsied sites that can mimic it (49).

Another problem was the high rate of false positive results regarding AIC as well as AF. Although there is an observed increase in detection rate, there were a non-negligible number of suspicious AIC and/or AF images that proved to be normal afterwards. The reason of false positive results with both methods is still unknown. A correct detection rate of 77.5% (31/40) for AIC was reported in a group of patients that were at that time morphologically non-suspicious but developed LC 14 months later (64). They concluded that AIC suspicious samples might be in an ongoing carcinogenesis process for a future, morphologically positive LC. Also, on following the outcome of a group of patients that were previously diagnosed as preneoplasia, it was found that previously classified images as AF suspicious, which proved morphologically negative at that time, developed morphologically positive preneoplasia later on (81). All these findings point out to the bad need of further genetic studies for deeper understanding of the carcinogenesis in bronchogenic carcinoma.

## **Summary**

**Background:** Conventional methods for early lung cancer detection as sputum cytology and white light bronchoscopy have a very low diagnostic rate not exceeding 40% and 29% respectively. Automated image cytometry and autofluorescence bronchoscopy are novel techniques developed to improve the diagnostic rate of such lesions with promising results.

**Objectives:** Can an increase in the diagnostic rate of ELC be achieved by combining AIC with WLB/AF examination?

**Patients and Methods:** In 1999-2000, 119 high-risk patients were recruited in 150 examinations in a case finding study. Initial investigations for presenting complaints were negative for lung cancer. All patients submitted airway secretion for AIC and conventional cytology (CY), underwent white light (WLB) and AF bronchoscopy. Suspected lesions were biopsied and examined histopathologically. Positive cases were followed up after 3 and 6 months.

**Results:** Based on histopathology and/or cytology, 23 preneoplasias (16 cases of moderate to severe dysplasia and 7 carcinomata in situ) were diagnosed. Although the use of AF, the preneoplastic lesions were not localized in 3 cytologically diagnosed cases out of 23. A relative sensitivity of WLB/AF to WLB proved to be 1.8. The sensitivity of all methods proved to be 100% (23/23) for detecting all preneoplasia cases. After excluding 9 non-Compliant patients from follow up analysis, a success rate of 12/14 cases (86%) in treating preneoplasia was achieved.

**Conclusion:** AIC of tracheobronchial secretions as well as AF are sensitive tools for the detection and localization of centrally located ELC lesions in a high-risk population. The complementary effect between both methods was represented as a 100% detection rate of all diagnosed preneoplasias.

**Recommendations:** Patients with positive AIC or AF bronchoscopy without histopathological or cytological evidence of preneoplasia should be followed up carefully as they may harbor a yet morphologically undetectable ELC. The cut off points of 2cDI in the evaluation of preneoplasia may have to be redefined and differentiated from that for invasive cancer. The previously mentioned scheme for preneoplasia management proved a success rate of 86% in its treatment. AIC of tracheobronchial secretions and WLB/AF proved to be useful, rapid, accurate and inexpensive methods that should be highly effective, in combination with other complementary methods, as a screening tool in asymptomatic, high-risk patients.

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